

# Diencephalic amnesia: Rodent cognition, neural markers and electrophysiology

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I declare that the work described in the current thesis has been done by myself, except where indicated.

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# Abbreviations

Ach	acetylcholine
ACC	anterior cingulate cortex
ANOVA	analysis of variance
ATN	anterior thalamic nuclei
Aud	auditory cortex
AV	anteroventral thalamic nucleus
B-L	bregma to lambda measurements
CA1-3	cornu ammonis area 1-3
c-AMP	cyclic adenosine mono-phosphate
CFC	cross-frequency coupling
Contra	Contralateral side
CO	cytochrome oxidase
CREB	c-AMP response element binding protein
DG	dentate gyrus
DTg	dorsal tegmental nuclei of Gudden
DHP	digital head stage processor
dHPC	dorsal hippocampus
EE	enriched housing
EEG	electroencephalogram
Fx	fornix
HPC	hippocampal formation
IEG	immediate early gene
IL	infralimbic cortex
ILN	intralaminar nuclei
Ipsi	ipsilateral side
KS	Korsakoff's syndrome
LFP	local field potential

LMB	lateral mammillary bodies
MB	mammillary bodies
MD	mediodorsal nucleus
MMB_L	medial mammillary bodies Pars Lateralis
MMB_M	medial mammillary bodies Pars Medialis
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
MS	medial septum
MTT	mammillothalamic tract
MTG	mammillotegmental tract
MWU	Mann-Whitney U test
NMDA	N-Methyl-D-Aspartate
pCreb	phosphorylated c-AMP response element binding protein
PD	Parkinson's disease
PL	prelimbic cortex
PFC	prefrontal cortex
pFx	post commissural fornix
post-sub	post subiculum
PSD	power spectral density
RAM	radial arm maze
Rdg	dysgranular retrosplenial cortex
Rga	granular a retrosplenial cortex
Rgb	granular b retrosplenial cortex
RSC	retrosplenial cortex
SD	standard deviation
SEM	standard error of the mean
SUM	supramammillary nucleus
Sup.	superficial layers
TBS	theta burst stimulation



WE	Wernicke's encephalopathy
VHPC	ventral hippocampus
VTg	ventral tegmental nuclei of gudden

## Abstract

The anterior thalamic nuclei (ATN), mammillary bodies and their interconnecting fibre tract the mammillothalamic tract (MTT) are important components of an extended hippocampal circuit for episodic memory. In humans, damage to the MTT or ATN from stroke, alcohol abuse, developmental brain injury and neurodegeneration is associated with severe anterograde amnesia. However, uncertainty regarding lesion boundaries and influence of pathology elsewhere in clinical settings limits firm conclusions regarding the specific contributions of the MTT and ATN to memory. These two structures are often considered to be functionally equivalent, but a direct comparison of ATN and MTT lesions in rats in Experiment 1 found that only ATN lesions impaired spatial reference memory and produced more severe deficits than MTT lesions on spatial working memory tasks in the radial-arm maze (RAM). ATN lesions also reduced *zif268* expression to a greater degree in the retrosplenial cortex and hippocampal CA1 than MTT lesions. However, MTT and to a lesser extent, ATN lesions reduced NeuN cell counts in all three regions of the MB, so the relative impact of these lesions is not explained by MB pathology. Rather the greater ATN lesions deficits found here likely reflect disruption to additional processes associated with dense connections of the ATN with both hippocampal and cortical sites. In a previous study, an unexpected discrepancy between ATN and MB lesions was found on an object-place paired-associate task. This anomalous finding was examined and resolved in Experiment 2. Rats with MTT lesions had impaired acquisition of object-place associations in which probe tests revealed that they were likely to be responding on the basis of location, not direction or egocentric information. Experiment 3 provided highly novel evidence that MTT lesions can disrupt rhythmic oscillatory interactions at theta frequency (4-12Hz) between the ATN-PFC, ATN-HPC and HPC-PFC while rats make spatial memory choices in the RAM. It would therefore be expected that ATN lesions may even more profoundly impair oscillatory activity within the circuit. These findings provide critical insight into two different perspectives regarding the association between diencephalic pathology and amnesia. Specifically, it appears that other direct and indirect cortical and hippocampal inputs of the ATN make it a relatively more critical point of dysfunction than would be expected by a primary focus on the albeit important VTg, MB and MTT brainstem inputs to the extended memory system.

# Chapter 1

## Introduction

This chapter provides an initial brief overview of the more detailed information presented in subsequent chapters. In the first section, “General Introduction”, each of the background information chapters in this thesis (Chapters 2-5) will be summarized. In the subsequent section, “Aims of the current study”, the main aims of the thesis will be stated and the experimental chapters (Chapters 6-8) will be introduced. The final section provides an “Outline of the thesis”.

### 1.1. General Introduction

Memory is central to our cognition, emotions and identity, and describes the brain’s ability to retain information and use it for adaptive purposes. Amnesia refers to a profound impairment in the ability to either form new memories (anterograde) or retrieve past memories (retrograde) in the presence of relatively preserved cognitive functions (Aggleton, 2014; Squire et al., 2011). Clinical examples show that damage to either the medial temporal lobe or the diencephalon can result in severe anterograde amnesia (Aggleton & Brown, 1999; Carlesimo et al., 2011; Kopelman, 2014). Considerable overlap in the memory impairments resulting from damage to these two distinct regions suggests that they work in parallel to support episodic memory (Delay & Brion, 1969; Aggleton, 2008; Aggleton, 2014). In their seminal review, Aggleton and Brown (1999) expanded on this idea and suggested that episodic memory and “recollection” rely on an extended circuit emanating from the hippocampus to include the anterior thalamic nuclei (ATN), the mammillary bodies (MB), the retrosplenial cortex (RSC), the prefrontal cortex (PFC) and two fibre tracts, the fornix (Fx) and the mammillothalamic tract (MTT). This perspective is strongly supported by an extensive animal literature examining the effects of lesions to the hippocampal formation as well as the nuclei and fibre tracts of the diencephalon (Aggleton, 2008; Aggleton et al., 2010; 2011, Aggleton & Nelson, 2015; Dalrymple-Alford et al., 2015). More recently, this neural network has been extended to include a unique brainstem input from the tegmental nuclei of Gudden (Dillingham et al., 2015; Vann & Nelson, 2015). Although the role of the hippocampal formation has dominated memory research since the 1950’s the association

between anterograde amnesia and damage to the diencephalon was recognised much earlier in the contexts of the Korsakoff syndrome (Kopelman, 2014; Aggleton, 2014).

Furthermore, recent evidence suggests that rather than a passive relay of hippocampal information sites within the diencephalon may actively regulate hippocampal function (Stypulkowski et al., 2014; Gibson et al., 2016). Within the diencephalon, damage to the mammillothalamic tract (MTT), anterior thalamus (ATN) and mammillary bodies (MB) are most often associated with anterograde amnesia (Harding et al., 2000; Van der Werf et al., 2000 & 2003; Carlesimo et al., 2011; Kopelman, 2014; Aggleton, 2014). Chapter 2 will expand on this idea of an extended hippocampal system and provide more in depth information about the structures and fibre tracts within this system referred to in the subsequent chapters describing the human and animal literature.

Although multiple nuclei and fibre tracts have been implicated in anterograde amnesia following damage to the diencephalon, pathology of the mammillary bodies or anterior thalamic nuclei, and the mammillothalamic tract between these two structures, is most consistently cited (Aggleton, 2014). Thalamic strokes that have produced relatively localised injury have provided the strongest evidence of MTT-related memory deficits. In a review that examined the largest number of thalamic stroke cases, interruption of the MTT alone was the most consistent predictor of an amnesic syndrome (Carlesimo et al., 2011). Evidence from diencephalic stroke has provided less consistent evidence for the involvement of thalamic nuclei, including the ATN (Van der Werf et al., 2003; Carlesimo et al., 2011). The strongest evidence for ATN involvement has come from the alcoholic Korsakoff's syndrome (KS), which is characterised by a dense amnesic syndrome following extended thiamine deficiency (Harding et al., 2000; Kopelman, 2014). Mammillary body atrophy is also a consistent finding in patients with the KS suggesting that MB pathology may also contribute to memory impairments (Kopelman 2014; Sechi & Serra, 2007), but MB pathology does not appear to be the critical substrate for amnesia in KS patients (Harding et al., 2000). Alternative evidence for MB-related memory deficits has come from a large cohort of patients that had colloid cysts removed from the 3<sup>rd</sup> ventricle (Tsvilis et al., 2008). Penetrating brain injuries also support a MB/MTT role in memory, although pathology often extends across multiple regions making definitive conclusions difficult (Squire et al., 1989; Dusior et al., 1990). Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis have also implicated the diencephalon. These neurological disorders have provided examples of diencephalic injury and memory loss embedded within a more complex

symptomology (Braak & Braak, 1991a,b; Rub et al., 2002; Houtchens et al., 2007). However, the frequent additional damage found in even the most localised clinical cases has limited firm conclusions regarding the specific contribution of diencephalic structures to memory.

Animal lesion studies have helped address the problem of lesion specificity. Given the consistent involvement of the MTT and ATN in human cases of diencephalic amnesia lesions to these two regions are the focus of the current thesis. Separate studies creating either MTT or ATN lesions have reported that lesions to either structure result in spatial working memory deficits in the radial arm maze and water-maze (Sziklas & Petrides, 1999; Van Groen et al., 2002; Vann & Aggleton, 2003; Vann, 2013; Vann & Nelson, 2015 Dalrymple-Alford et al., 2015). ATN lesions have also been shown to severely impair spatial alternation in a T-maze, with less consistent deficits found following MTT lesions (Aggleton & Vann, 2003; Vann, 2013; Frizzarti et al., 2016).

One possible point of difference between MTT and ATN lesions relates to aspects of place learning. ATN lesions consistently produce profound impairments on the standard reference memory version of the water maze, whereas MTT lesions produced only a mild and transient deficit that recovered by the final day of testing (Warburton et al., 1999, Warburton & Aggleton, 1999; Wilton et al., 2001; Wolff et al., 2008a; 2008b; Winter et al., 2011; but see Sutherland & Rodriguez, 1989). It also appears that rats with MTT lesions are able to make a relative geometric discrimination to locate a hidden platform (Vann, 2013), but rats with ATN lesions discriminated at the level of chance on this same task (Aggleton et al., 2009; Dumont et al., 2014). Despite these separate studies hinting at functional dissociations between these two structures, the limited MTT literature, and especially the lack of any direct lesion comparison to avoid subtle procedural differences between studies, has restricted definitive conclusions regarding the relative impact of MTT and ATN lesions on memory.

Another interesting feature of MTT and ATN lesions is that they consistently have functional effects on other distal regions of the extended hippocampus system. The retrosplenial cortex is especially sensitive to ATN and MTT lesions, showing substantial lesion induced hypoactivation of neural activity markers such as the immediate early gene (IEG) products c-fos and zif268 (Jenkins et al., 2002; Vann & Albasser, 2009; Dumont et al., 2012; Dupire et al., 2013; Frizzarti et al 2016; Loukavenko et al., 2016). Such ‘functional lesions’ are thought to contribute to the severity of lesion related memory deficits (Aggleton, 2008; Aggleton & Nelson, 2015; Dillingham et al., 2015). Both MTT and ATN lesions have

resulted in less consistent IEG changes in the hippocampus suggesting these effects may be task specific (Jenkins et al., 2002a,b; Vann & Albasser, 2009; Dupire et al., 2013; Vann 2013, but see Dumont et al., 2012; Loukavenko et al., 2016; Frizzarti et al., 2016). Although patterns of c-fos and zif268 hypoactivation following MTT and ATN lesions appear to be similar a direct within study comparison is needed. Such evidence would provide further information regarding the relative impact of lesions to the MTT and ATN.

A more direct way of determining the influence on diencephalic lesions on the extended hippocampal system is examine real-time interactions by recording electrophysiological activity within key structures of the circuit especially the HPC and mPFC. Rhythmic oscillatory activity is generated by the cumulative electrical currents from all excitable membranes, and this activity often oscillates in waves of varying frequency and amplitude (Buzsaki et al., 2012). Synchronisation of this rhythmic activity across structures, also known as coherence, is proposed to be indicative of functional inter-dependence and may increase during complex cognitive tasks (Fell & Axmacher, 2011; Colgin, 2011). Of the various oscillatory bands theta (4-12 Hz, sometimes referred to as theta at 4-7 Hz and alpha at 8-12 Hz) and gamma (30-100 Hz) have received the greatest attention in memory research (Buzsaki, 2002; Nyhus & Curran, 2010; Colgin, 2013). It has been proposed that whilst theta can coordinate long range activity, fast oscillations such as gamma are important for local processing and may be used to represent discrete units of information (Nyhus & Curran, 2010). Both human and animal studies have suggested an important role for theta and gamma rhythmicity in successful memory processing and aberrant activity occurs in disease states associated with impaired cognition (Tort et al., 2009; Nyhus & Curran, 2010; Shirvalkar et al., 2010; Colgin, 2011, 2013; Palop & Mucke, 2016).

In rodents, spatial memory performance is consistently associated with increased theta synchrony between the hippocampus and the prefrontal cortex (Jones & Wilson, 2005; Hyman et al., 2010; Kim et al., 2011; O'Neill et al., 2013). Theta rhythm is ubiquitous throughout the structures of the extended memory network, although the source and role of rhythmic activity propagated by the diencephalon is still not well understood (Kirk et al., 1996; Basset et al., 2001; Kocsis & Vertes, 2001; Kirk and Mackay, 2003; Ketz et al., 2015). One view suggests that the rhythmicity of the diencephalon provides a mechanism for aligning activity within the diencephalon, hippocampus and neocortex during mnemonic processes (Kirk & Mackay, 2003). An alternate perspective suggests that the ventral

tegmental nucleus provides an additional theta input to the ATN via the MB/MTT that may be fundamental to hippocampal rhythmicity (Bassant et al., 2001; Kocsis & Vertes, 2001). Regardless of its source these theories suggest that damage to the diencephalon might in part reflect disrupted coupling between the RSC, HPC and mPFC from a loss of ascending rhythmic input via the ATN. Such a scenario might also explain why approximately 75% of patients with alcoholic Korsakoff syndrome recover if they remain abstinent and how enriched housing can improve spatial memory deficits in rats with ATN lesions (Kopelman et al., 2009; Loukavenko et al., 2007, 2016; Wolf et al., 2008; Harland et al., 2014). That is, although permanent damage is apparent in the diencephalon the ‘functional’ lesions in the HPC, RSC and PFC may remain amenable to intervention. Thus increasing or normalising activity within these regions may be able to somewhat compensate for the loss of diencephalic input. For example, models of thiamine depletion in rats have shown that upregulation of cholinergic activity in the hippocampus, prefrontal cortex or medial septum (all strongly implicated in theta rhythmicity) can improved memory function (Savage et al., 2012). It is therefore highly relevant to determine the effect of MTT lesions on activity upstream in structures such as the ATN, HPC and PFC.

## 1.2. Aims of the current study

This brief introduction, elaborated in subsequent chapters, points to two separate perspectives regarding the critical site of pathology in diencephalic amnesia. One perspective supported by cases of thalamic stroke, emphasises the importance of the brainstem input to the extended hippocampal circuit via the MTT. An alternative perspective, supported by neuropathology in Korsakoff syndrome, suggests that the ATN forms a critical node within this circuit because of its dense connections with subcortical and cortical sites. Studies examining neural activity and plasticity markers suggest that ‘functional lesions’ occur in distal regions including the hippocampus, prefrontal cortex and retrosplenial cortex following damage to the diencephalon. These changes indicate dysfunction within isolated structures, but cannot provide information regarding altered communication within the circuit. One possibility is to examine the rhythmic oscillatory activity in the extended hippocampal network. Slow wave oscillations such as theta are of particular relevance because of its known role in coordinating activity between the hippocampus and prefrontal cortex during mnemonic processes.

The aim of the current thesis was to examine the behavioural, neural and electrophysiological impact of MTT lesions. Moreover, the study provides insight into two different perspectives regarding the critical site for diencephalic amnesia by providing the first direct comparison of MTT and ATN lesions within a study. The study also extended previous MTT lesion findings beyond standard spatial working memory tasks by testing rats on an object-place paired associate task, which is particularly relevant to human memory and clinical amnesia. A major aim of this study was to provide highly novel insight into the impact of MTT lesions on rhythmic oscillatory activity both within and between critical structures within the extended hippocampal circuit. In addition, parameters for electrical stimulation of the ATN were explored that could be used to enhance spatial memory performance in rats with MTT lesions. These objectives were accomplished across three empirical studies.

The first experiment investigated the relative behavioural impact of MTT and ATN lesions, as well as lesion-related changes in the expression of the immediate early gene *zif268* in cortical structures and cell counts in the MB. Prior to surgery, rats were trained for 12 days on the standard working memory task in the radial arm maze, performance on which was used to assign matched pairs of rats to ATN lesions, MTT lesions or Sham ATN or MTT surgery conditions. After receiving lesion or sham surgery, the rats were tested on a standard reference memory task in the water maze. Next, all rats were tested on the standard working



memory task in the water maze, followed by a version when allocentric information was minimised. MTT lesion and Sham lesion groups were then examined to determine whether the MTT contributes to object-place paired associate learning (experiment 2). The groups were then tested on the standard RAM task, followed by a mid-trial delay and mid-trial delay + rotation RAM tasks. To induce the expression of zif268 rats received three days of massed trials on the mid-trial delay + rotation task in the RAM followed by 90 mins in a dark quiet room. This task is particularly sensitive to retrosplenial cortex damage, a site consistently associated with functional pathology following MTT and ATN lesions, making functional changes behaviourally relevant (Pothuzian et al., 2008; Poirier & Aggleton, 2009; Vann & Albasser, 2009; Dumont et al., 2012; Frizzarti et al., 2016). Zif268 positive cell counts were quantified in all sub-regions of the RSC, the anterior cingulate, the hippocampus and a cortical control region. Furthermore, anecdotal reports suggested that both MTT and ATN lesions result in MB cell loss (Aggleton & Mishkin, 1983; Vann & Aggleton, 2003; Vann, 2013). This observation was formerly tested by quantifying the expression of the neural specific marker NeuN in the three regions of the MB.

Previous work has suggested that ATN lesions profoundly impair object-place paired associated learning (Sziklas & Petrides, 1999 & Dumont et al., 2014). By contrast, lesions to the mammillary body region acquired object-place pairings at an equivalent rate to controls (Sziklas et al., 1996) This finding provides an anomaly within the literature and implies a very clear distinction between ATN and MB lesions, which therefore needs revisiting. In the current thesis the MTT and MTT sham rats had to learn that one of two distinct objects (presented simultaneously) was rewarded when placed in the East end of a large diamond arena, and the alternate object was correct when placed in the West end. Rats were then tested on three separate probe trials to examine what strategies they were using to perform the task.

The third experiment examined whether dysfunctional rhythmic activity within the extended hippocampal circuit is associated with MTT lesions. Rats were randomly allocated to MTT or MTT sham lesions and subsequently tested on the standard RAM task to establish a lesion deficit. All rats then had microwire recording electrodes implanted in the prefrontal cortex, ATN and the hippocampus. At the end of the surgery recordings were taken for each rat during baseline and then with a tail pinch. The rats were then run on a random foraging task in a large activity box to drive rhythmic activity. Spatial memory with electrophysiological recording was then taken in the radial arm maze, first in the standard RAM task and then on a mid-trial delay RAM task. To introduce greater temporal specificity

over the electrophysiological recording infra-red beam breaks were added to the maze and the rats received additional testing on the standard RAM task. A preliminary investigation in to the efficacy of theta burst stimulation of the ATN to promote recovery of function was then explored. ATN stimulation has been shown to result in improved cognitive performance in humans and rats (Oh et al., 2012; Hamarni et al., 2010). Furthermore, ATN stimulation has been shown to directly alter activity in the hippocampus and prefrontal cortex (Stypulkowski et al., 2014; Gibson et al., 2016). The electrophysiological impact of ATN TBS-stimulation on the hippocampus and prefrontal cortex was first assessed in a standard cage, before being applied to rats during their first 4 arm choices in the RAM. At the end of the experiment zif268 activity was driven by unilateral stimulation of the ATN, so stimulation related zif268 changes could be examined across hemispheres in the hippocampus and retrosplenial cortex.

### 1.3. Outline of thesis

The following chapter introduces the concept of an extended hippocampal system and briefly describes its structures with particular emphasis on the MTT, ATN and MB. Chapter three provides an introduction on the involvement of the mammillothalamic tract and anterior thalamic nuclei in diencephalic amnesia by examining human clinical data. Chapter four discusses animal models of memory deficits and provides more precise experimental evidence on the involvement of the mammillothalamic tract and anterior thalamic nuclei in memory function. Chapter five examines the evidence that rhythmic oscillatory activity supports the interactions necessary for mnemonic processes within the extended hippocampal system. This chapter also discusses evidence that impaired memory following diencephalic damage may in part reflect altered rhythmicity between regions such as the prefrontal cortex and hippocampal formation.

The subsequent three chapter (Chapters 6-8) provide details on the methods and results of the three experiments described above. Chapter nine provides a general discussion of the experimental and theoretical relevance of the findings of the current research in the context of the literature reviewed in chapters 2-5. The limitations of the current study, as well as suggestions for future directions in research are also discussed towards the end of chapter nine.

## Chapter 2

# Neuroanatomy of the extended hippocampal system

This chapter provides a more detailed examination of the brain areas involved in episodic memory that are relevant to this study. A general overview of the extended hippocampal circuit is described first, before a more detailed description of the key components of this circuit. There is a particular focus on the mamillothalamic tract, mammillary bodies and anterior thalamus because of their consistent implication in human anterograde amnesia. Anatomy and relevant connections will be discussed as well as relevant differences between rat and primate neuroanatomy.

### 2.1. The extended hippocampal circuit

The idea of an extended neural circuit centring on the hippocampal formation is not new. Credited to James Papez in 1939 (but perhaps first described by Jakob, see Triarhou, 2008), the ‘Papez circuit’ was proposed to provide the basis for emotional experience. This circuit was deemed to originate in the cortex, then “built up in the hippocampal formation and... transferred to the mammillary body and thence through the anterior thalamic nuclei to the cortex of the gyrus cinguli” (Vann & Nelson, 2015). Despite the Papez circuit’s assumed role in emotional experience, the consistent implication of its constituent structures in human amnesia suggested this circuit might instead be more relevant to memory. More recent descriptions have built on this idea, first as the Delay and Brion memory circuit (1969) and more recently as an explicit extended hippocampal circuit responsible for recollective aspects of memory (Aggleton & Brown 1999). In the latter formulation, the extended hippocampal (figures 2.1 & 2.2) centers on the hippocampal formation (HPC) and its connections with the retrosplenial cortex (RSC), and prefrontal cortex (PFC), the anterior thalamus (ATN) and mammillary bodies, along with an emphasis on the fornix (Fx) in addition to the mamillothalamic tract (MTT) (Aggleton & Brown 1999; Vann & Nelson 2015). The fornix provides a major bidirectional connection between the hippocampal formation and the diencephalon, which is reciprocal in the case of the ATN and an afferent projection only to

the mammillary bodies (the descending postcommissural fornix). In turn, the MTT provides a unidirectional efferent connection from the MB to the ATN, thus providing an additional indirect hippocampal input to the ATN. The MTT are unusual by virtue of being the only structure that is unique to the memory circuit. That is, via their MTT efferents, the MB provide a unique brainstem influence into the ATN and hence a wider system that includes the brainstem tegmental nuclei of Gudden via tracts in the mammillary peduncle (Vann & Nelson, 2015).

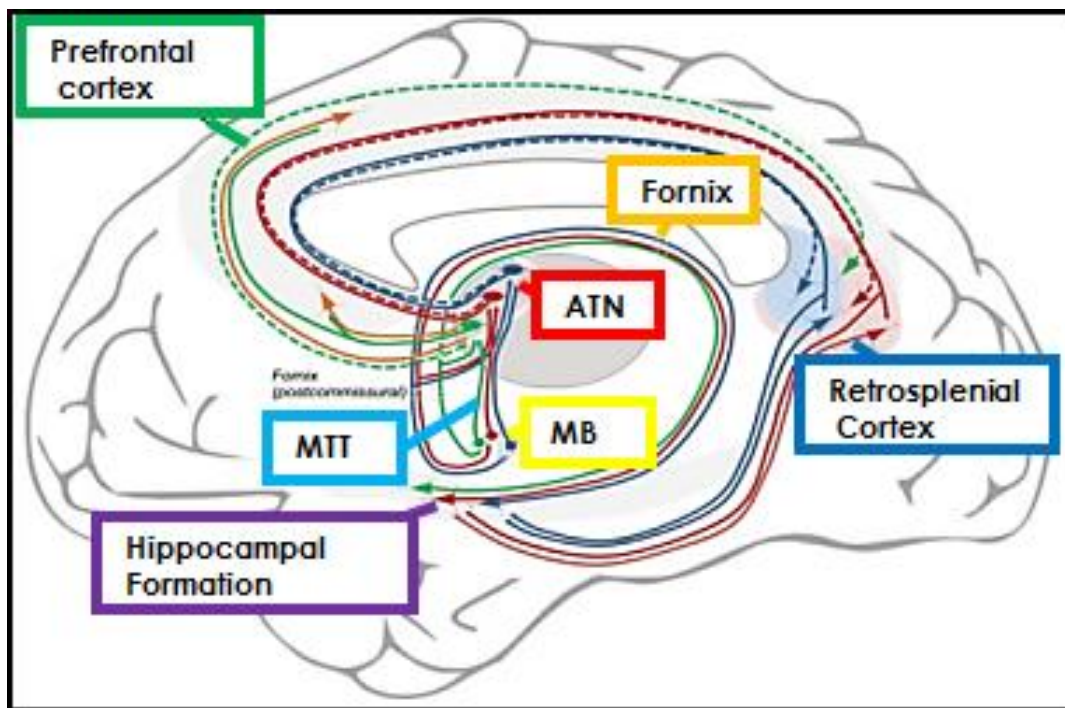


Figure 2.1: Diagrammatic representation of the major components of the extended hippocampal memory circuit in a human brain, adapted from Child and Bennaroch (2013).

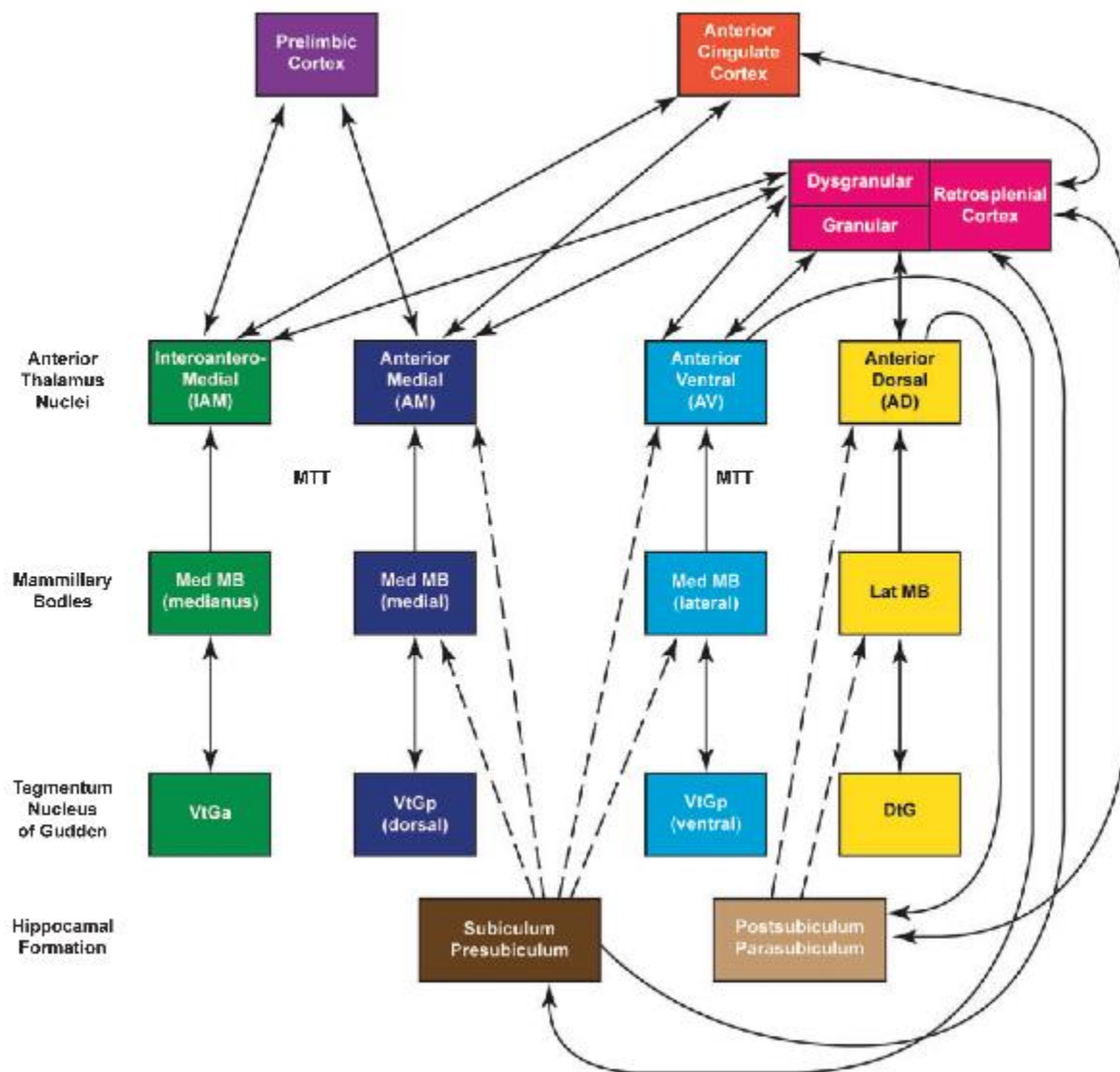


Figure 2.2: From Aggleton et al. (2010), diagram of the interlinked connections constituting the extended hippocampal system in the rat brain. Connections provided by the fornix are represented by dashed lines and double headed arrow indicate reciprocal connections.

**Abbreviations:** DtG, dorsal tegmental nucleus of Gudden; MTT, mammillothalamic tract; VtGa, ventral tegmental nucleus of Gudden, pars anterior; VtGp, ventral tegmental nucleus of Gudden, pars posterior.

## 2.2. The mammillary bodies (MB)

The MB represent a prominent location on the ventral surface of the brain just posterior to the hypothalamus. Each side of the bilateral MB consists of two main subregions, the medial MB nuclei and lateral MB nuclei. The medial MB can be further subdivided into the pars lateralis and pars medialis (Vann, 2010). Differences in cell morphology are apparent between the medial MB nuclei and the lateral MB nuclei, but there only appears to be one cell type within each subnucleus (Veazey et al. 1982). In addition, in rodents all the cells in the MB appear to be projection cells, with the distinct absence of interneurons (Takeuchi et al., 1985; Veazey et

al., 1982), supporting the position that the MB primarily represent an important relay within the circuit (Hopkins, 2005). However, there is evidence suggesting interneurons may be present in higher order species such as humans and nonhuman primates (Bernstein et al., 2007; Dixon et al., 2004).

The MB are different to the other structures in the extended hippocampal circuit in that they have major connections with only a limited number of sites. The two primary inputs to the MB are from the hippocampal formation (HPC via the descending component of the postcommissural fornix (pF<sub>x</sub>) and from the tegmental nuclei of Gudden via the mammillary peduncle (Dillingham et al., 2015). In turn, the MB appear to project almost exclusively via the MTT to the ATN and send a reciprocal projection to the tegmental nuclei of Gudden via the mamillo- tegmental tract (Dillingham et al., 2015). The lateral MB and medial MB nuclei project to different sub regions of the ATN, which are described as two parallel systems, one a medial ‘theta’ system and one a lateral ‘head direction’ system (Vann & Aggleton, 2004; Aggleton et al., 2010). Arising from the HPC, the medial MB are innervated by projections in the dorsal, ventral and intermediate subiculum and the medial entorhinal cortex; the lateral MB receive inputs from presubiculum, parasubiculum and postsubiculum. Although the ATN is also densely and directly innervated by the subiculum, the subicular cells projecting to the ATN originate from the deepest cell populations, whereas those projecting to the MB derive from more superficially located cells (Wright et al., 2010). We do not yet know whether these different subicular neurons convey parallel or independent information to the MB and ATN. In terms of hemispheric MB connections, the medial MB project ipsilaterally to the anteromedial and anteroventral thalamic nuclei whereas the lateral mammillary nuclei have a degree of bilateral projections to the anterodorsal thalamic nuclei (Cruce, 1975; Dillingham et al., 2015). The medial MB have reciprocal connections with the ventral tegmental nucleus of Gudden (VTg), whereas the lateral MB have reciprocal connections with the dorsal tegmental nuclei of Gudden (DTg) (Dillingham et al., 2015).

### 2.3. The mamillothalamic tract (MTT)

Unlike the fornix, the fibres of the MTT contained entirely within the extended hippocampal circuit (Vann, 2010). For this reason it has been suggested that, understanding the functional contribution of the MTT may provide critical information about how the constituent structures interact as a circuit to support episodic memory function (Vann & Aggleton, 2003).

The efferent fibres of the MTT project from both the medial and lateral mammillary nuclei, up through the hypothalamus to innervate all three elements of the anterior nuclei of the thalamus (Powell et al., 1957; Cruce, 1975; and Seki & Zyo, 1984). As such, the MTT is proposed to be functionally equivalent to the mammillary bodies and is thought to support ATN function by providing an additional hippocampal input, as well as a unique brainstem input into the circuit (Vann & Nelson, 2015; Dillingham et al., 2015).

## 2.4. The anterior thalamic Nuclei (ATN)

The ATN of the rat is divided into the anterodorsal, the anteroventral and the anteromedial nuclei, all located in the rostral dorsomedial thalamus (Jankowski et al. 2013). The ATN sits as nodal point within a complex array of cortical and subcortical connections (Aggleton et al., 2010; Dalrymple-Alford et al., 2015). These include widespread links to the frontal cortex, much of the cingulate cortex but especially the retrosplenial cortex, and the hippocampal formation. Many of these connections are reciprocal (Shibata & Naito, 2005). Especially dense inputs to the ATN arise from the RSC and the subicular cortex, whereas there appear to be no direct connections with the hippocampus proper. It is interesting that almost every neuron within the MB projects to the ATN (Hopkins, 2005; Vann et al., 2007) suggesting that MB neurons provide critical information to the extended hippocampal system via their influence on the ATN. In addition, the anteroventral (AV) and anteromedial (AM) nuclei receive projections from different populations of subicular cells (Wright et al 2013). Recent evidence has suggested an asymmetric organization of anteroventral and anteromedial efferents to the prelimbic cortex, anterior cingulate cortex, retrosplenial cortex and subiculum. That is, the AV and AM provide ipsilateral projections to these cortical and hippocampal sites, whereas these structures tend to provide bilateral projections back, which appears to be especially true for the anteromedial nucleus (Mathiasen et al., 2017).

As indicated earlier, differences in connectivity have been tied to functional distinctions between the three subregions of the ATN. The anterodorsal nucleus is proposed to form a lateral head direction circuit for spatial navigation, together with the lateral mammillary bodies and the dorsal tegmental nucleus of Gudden (Vann & Aggleton, 2004; Aggleton et al., 2010; Jankowski et al., 2013). These structures contain a large proportion of head direction cells, which are only active when the animal is oriented in a specific direction, operating like a compass and thus thought essential to spatial navigation when head direction



is critical (Jankowski et al., 2013). Lesion and electrophysiological studies suggest that the head direction signal is generated by the reciprocal connections between the dorsal tegmental nuclei and the lateral mammillary bodies and then propagated up to cortical regions via the anterodorsal nuclei (Dillingham et al., 2015).

By contrast, the anteroventral and anteromedial nuclei are proposed to form part of a medial theta circuit, along with the medial mammillary bodies and the ventral tegmental nuclei of Gudden, although the anteroventral nucleus also contains some head direction cells (Aggleton, et al., 2010; Tsanov et al., 2011a,b). Theta activity in awake behaving rats is a slow wave rhythmic oscillation of the local field potential between 4-12 Hz (see Chapter 5). Within the hippocampal-diencephalic circuits theta rhythm is thought to play a critical role in synchronizing distally located populations of neurons. Therefore theta rhythm has been proposed to provide a framework for the inter-structural communication necessary for complex cognitive functions such as memory processing (Buzsaki, 2002, 2005; Kirk & Mackay, 2003; Rutishauser et al. 2010; Colgin, 2011).

## 2.5. The hippocampal formation (HPC)

The HPC is a subcortical structure located within the medial temporal lobe bordering the rostral brainstem. This important structure within the limbic system is involved in the consolidation of new information and spatial navigation (Buszaki 2005; Aggleton 2012). The HPC consists of several subregions including the CA1-4 fields, dentate gyrus and the subicular complex (Squire et al. 2004). The primary efferent projections from the HPC emanate from CA1 and the subicular cortices. Connections with the ATN, MB and RSC all originate from the subiculum and presubiculum, not the CA fields of the hippocampus proper (Aggleton, 2012). As indicated earlier, these efferents arise from separate lamina within the subiculum, so that the ATN, MB and RSC receive input from non-overlapping populations of cells. Moreover, different subicular regions along the rostral caudal axis terminate in specific parts of the ATN, MB and RSC (Aggleton 2012). By contrast the hippocampal efferents to the prefrontal cortex involve a more equal balance of cells from the subiculum and CA1. Importantly, the ATN and RSC also project back upon the hippocampal formation providing both direct fornical and indirect cortical pathways between the HPC and diencephalon creating a circuit.



## 2.6. The retrosplenial cortex (RSC)

The term ‘retro-splenial’ defines the RSC’s position immediately behind the splenium of the corpus callosum in primates (Vann et al., 2009). In human and nonhuman primates the RSC is defined posterior to the cingulate cortex as areas 29 and 30. In the rodent, the posterior cingulate region differs substantially from the primate as the entire posterior cingulate region is designated RSC and makes up a substantial proportion of cortical tissue (Vann et al., 2009). The RSC is divided into the following subregions: the granular RSC consisting of the more caudally placed granular a region (Rga), the more rostral granular b (Rgb) region (area 29), and the dysgranular RSC (Rdg; area 30). The Rga, Rgb and Rdg are interconnected, but have different extrinsic connections. The granular regions have considerable inputs from the temporal and septal subiculum, whereas the Rdg has dense connections with the visual system (Shibata et al., 2009). The association of the RSC with learning and memory grew primarily from its dense reciprocal connections with the HPC (subiculum, presubiculum and parasubiculum) and the ATN. Given its anatomical position, the RSC provides a major cortical junction for interactions between HPC and ATN. This convergence provides a possible explanation for the similarity of the deficits produced by medial temporal lobe and diencephalic amnesia which may have their basis in the joint covert dysfunction of the retrosplenial cortex after injury to these brain regions displayed primarily by profoundly reduced markers of neural activity in the RSC (Aggleton, 2008; Dillingham et al., 2015). This position is further supported by evidence that the RSC shows pathology and metabolic decline early in the progression of Alzheimers disease (Aggleton et al., 2016).

## 2.7. Prefrontal cortex (PFC)

The PFC is a large and diverse cortical region that occupies the anterior portion of the frontal lobe (Ongur & Price, 2000). The PFC as a whole receives multiple streams of processed sensory information, and is involved in high level cognitive and emotional processes (Ongur & Price, 2000). The PFC shows considerable variation across species, especially regarding the amount of granular vs agranular cortex present, but there are also considerable similarities in the position and connection of its subregions across species. In terms of the extended hippocampal circuit, the ventral medial prefrontal cortex (mPFC) is of particular note. The ventral mPFC includes the prelimbic and infralimbic cortex which are densely innervated by the HPC (CA1 and the subiculum), but also receive moderate projections from the ATN and

RSC (Hoover & Vertes, 2007; Mathiasen et al., 2017). Of relevance to the current thesis, electrophysiological studies suggests that interactions between the HPC and the ventral mPFC play a critical role in memory processing (Colgin, 2011)

## 2.8. The fornix (Fx)

The Fx is a major bundle of fibres that forms a vital connection between the medial temporal, medial diencephalic and brainstem regions. Central to the “extended hippocampal” memory model is the notion that the direct projections from the hippocampal formation to the ATN and MB, via the Fx, are critical for memory (Aggleton, 2014). At the level of the anterior commissure, approximately half of the fibres in the fornix continue forward to form the precommissural fornix (Nauta, 1956; Poletti & Creswell, 1977; Powell et al., 1957; Raisman et al., 1966; Simpson, 1952) which innervate the basal forebrain (including septum), ventral striatum, and prefrontal cortex (Nauta, 1956; Poletti and Creswell, 1977). The precommissural fornix also contains the dense projections from the septum to the hippocampus (Votaw & Lauer, 1963). The other half the fibres in the fornix are relevant to the functions of the Papez circuit, within the post commissural fornix, approximately one half to two thirds of which do not reach the mammillary bodies (Powell et al., 1957). That is, a large component of the post-commissural fornix directly innervates the ATN while the remaining fibres then descend to innervate the MB (Guillery, 1956) among other regions such as the nucleus reuniens and the supramammillary nucleus (Kishi et al. 2000; Sprague & Meyer, 1950). As summarised in Chapter 4, lesions to the caudal descending post-commissural fibres that project directly to the MB appear to have limited behavioural consequences on standard memory tasks that are impaired by MB and MTT lesions (Vann et al., 2010; Vann, 2013). The latter evidence points to a strong influence on the extended hippocampal system via brainstem connections with the MB.

## 2.9. The tegmental nuclei of Gudden

More recently, the tegmental nuclei of Gudden have been added to the extended hippocampal system in recognition of the unique influence they provide to the circuit via the MB and MTT. Behavioural studies in rats suggest that the ventral tegmental nuclei of Gudden (VTg), and not descending post-commissural fibres from the hippocampal formation, primarily drive

MB function (Vann, 2009; Vann et al., 2010; Vann, 2013). The tegmental nuclei of Gudden are located in the medial brain stem and as mentioned above provide one of the principal inputs into the mammillary bodies (Allen & Hopkins, 1989). The VTg has dense reciprocal connections with the medial MB, while the dorsal tegmental nucleus of Gudden (DTg) is densely interconnected with the lateral MB. Of particular relevance to the current thesis, cells in the VTg fire rhythmically in theta bursts which suggests a role in the modulation of theta activity in the extended hippocampal circuit (Kocsis et al., 2001; Bassant & Poindessous-Jazat, 2001). By contrast, the DTg contains head direction and angular-velocity cells (Bassett & Taube, 2001; Bassett et al., 2007). Consequently, it has been proposed that these two systems constitute the two parallel mammillary body systems i.e. the ‘lateral’ head direction system and the ‘medial’ theta system.

## 2.10. Summary

The structures described in this chapter together constitute a distributed neural network deemed critical for the acquisition of episodic memory. Despite the focus on the HPC in the literature, the MTT and ATN are also of note because damage to these latter two regions is consistently associated with anterograde amnesia in humans and MTT and ATN lesions result in severe spatial memory deficits in animals (Chapters 3 and 4). Furthermore, the MTT convey a unique brainstem influence from the MB to the rest of the extended hippocampal circuit via the ATN. In turn, the ATN is suggest to provide a critical nodal point for the exchange of subcortical and cortical information necessary for memory processing. Subsequent chapters present evidence from both human and animal studies and build a case for a direct comparison between MTT and ATN lesions, and explore the role of the diencephalon in the rhythmic oscillatory activity propagated throughout the extended hippocampal circuit.

## Chapter 3

# Diencephalic Amnesia

This chapter reviews the literature associating brain injury and various neuropathological conditions with diencephalic amnesia and makes a case for the involvement of the mammillothalamic tract, the anterior thalamus and the mammillary bodies. Although human cases rarely have damage limited to single structures, many clinical studies have highlighted a potential role for individual diencephalic structures, and have indicated discrete areas for animal lesion studies. The following section examines human case studies of diencephalic amnesia. Thalamic lacunar strokes with relatively localized injury provide the strongest evidence of MTT-related memory deficits, but less consistent evidence for the involvement of additional thalamic nuclei, including the ATN. Korsakoff's syndrome is a neurological disorder associated with chronic alcohol abuse and thiamine deficiency, characterized by a dense amnesic syndrome, which is associated with damage to the anterior thalamic nuclei and mammillary bodies. Thalamic cysts and penetrating brain injuries can also produce a varying degree of amnesia depending on the number and nature of brain regions affected. Neurodegenerative diseases also affect the diencephalon, and provide examples of diencephalic injury and memory loss embedded within more complex symptomology.

### 3.1. Thalamic infarction

The strongest support for the role of the MTT and to a lesser extent the ATN in diencephalic amnesia has come from cases of relatively selective diencephalic lacunar stroke (Van der Werf et al., 2003; Carlesimo et al 2011). Unlike haemorrhagic stroke, ischemia-associated lacunar stroke refers to occlusions of the penetrating arteries supplying blood to deep regions of the brain. The ventral and anterior thalamus is principally supplied by the tuberothalamic (polar) and paramedian arteries (see figure 3.1). The polar artery typically has a more anterior distribution although there is substantial neuroanatomical variability between individuals and the paramedian artery supplies the anterior region of the thalamus in about a third of cases (Von Cramon et al., 1985; Carrera & Bogoussalavsky, 2006). Infarcts in the anterior thalamic territory which involve the ATN and MTT account for approximately 12% of thalamic infarcts (Carrera & Bogoussalavsky, 2006). Lacunar stroke cases offer an advantage over other neurological and neurodegenerative diseases, such as Korsakoff's syndrome, in that

they are less likely to be accompanied by widespread pathology and provide a clearer association between structural damage and loss of function.

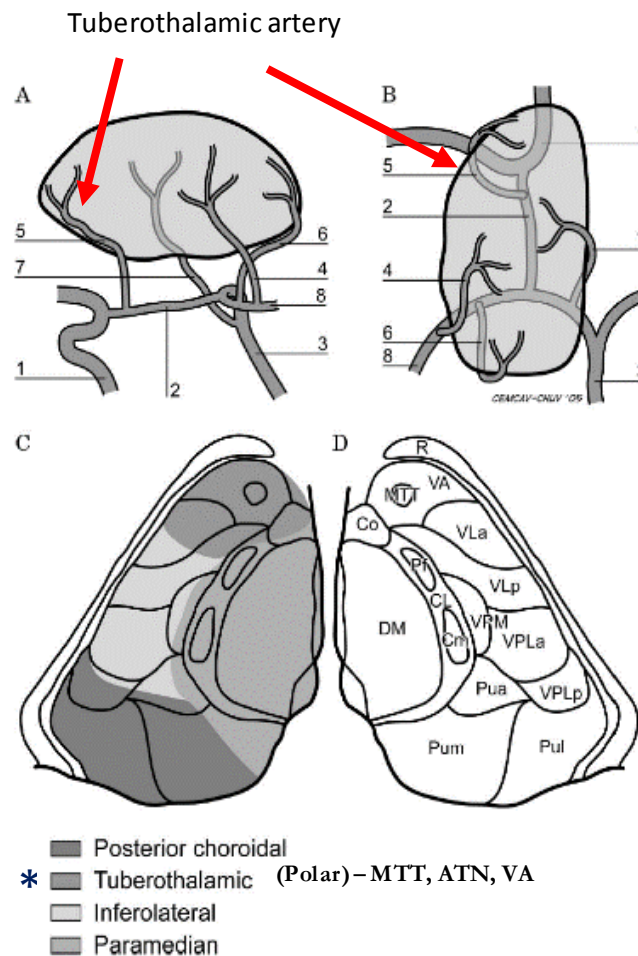


Figure 3.1: From Carrera and Bogoussalavsky (2006): location of the main arteries supplying blood to the thalamus from a lateral view (A) a dorsal view (B) and relationship between arterial territories and nuclear subgroups within the thalamus. The Tuberothalamic or Polar artery (red arrows) commonly supplies the anterior portion of the thalamus containing the MTT, ATN and ventral anterior nucleus.

An early study by Von Cramon et al., (1985) based on CT images of six patients with ventral thalamic infarcts suggested that damage to the MTT and the ventral part of the lamina medullaris interna was critical to the development of an amnesic syndrome. By contrast damage to the mediodorsal nucleus in the absence of pathology to other structures did not result in amnesia.

Subsequently a review by Van der Werf, et al., (2000) examined whether a relationship existed between neuropsychological consequences and damage to specific thalamic structures. Van der Werf et al. (2000) reviewed 35 articles which described a total of

60 patients with lacunar infarcts all within the boundaries of the thalamus. Across all of these cases the only clear structure function relationship was between an amnesic syndrome and interruption of the MTT. In fact 24 out of the 25 patient's suspected of having an amnesic syndrome had damage to the MTT. No clear relationships were found for non-amnesic memory problems or executive dysfunction.

More recently Van der Werf et al., (2003) included a cohort of 22 new cases of thalamic infarction recruited over four years and found a strong association between MTT damage and dense amnesia. In this study thalamic infarctions were confirmed with MRI imaging and extensive neuropsychological measures were taken for each patient. To try and establish clear structure function relationships a lesion overlap analysis was used. This analysis involved compiling the lesions of patients who shared a certain deficit then correcting these with the lesion distribution in patients without such deficits to determine remaining regions of interest. The association between MTT injury and amnesia was extracted from ten of the 22 cases described by Van der Werf et al., (2003) who were judged both to have 'clean' thalamic lesions without additional pathology such as additional infarctions or severe hippocampal atrophy and to be cognitively intact to satisfy criteria for a possible amnesic syndrome. Within this group, three cases had a classic amnesic syndrome and had damage to the MTT within the left (one case) or bilateral ventral thalamus. As an example, patient 9a from this subgroup had bilateral interruption of the MTT and presented with severely impaired verbal recall, verbal recognition and visual memory. Underlying the difficulty with clinical examples, however, other amnesic patients had additional deficits in executive function and speeded information processing, despite normal language skills, perception and IQ, and thus memory deficits may also be associated with non-MTT injury. Similarly, the association between dense memory loss and MTT injury was also found to overlap with damage to the ventral mediodorsal nucleus and the intralaminar nuclei. When Van der Werf et al., (2003) included patients with more widespread pathology there were a further four that suffered from amnesia and all of these cases had lesions encompassing the MTT. Van der Werf (2003) further suggested that as the MTT contains fibres bound for the ATN, it could be expected that infarctions affecting the ATN produce the same deficit as damage to the MTT. However, ATN-localised infarctions are rarely encountered (but see Parkin et al., 1993). No simple relationships were found between other thalamic structures, memory, executive functioning or attention.

A subsequent review by Carlesimo, Lombardi & Caltagirone (2011) extended the work of Van der Werf et al. (2003) by including a larger sample of patients and also particular emphasis to the type of declarative memory deficits. These authors reviewed 41 papers published between 1983 and 2009. These studies provided data on a total of 83 patients with lacunar infarcts in the mesial and anterior regions of the thalami, both frequently associated with amnesic syndromes. Nearly all the patients with a neuroradiologically documented lesion to the MTT presented with a clinically relevant memory disorder confirmed by formal neuropsychological evaluation. Carlesimo et al's (2011) study also lends support for an extended hippocampal system as they found that the qualitative pattern of memory impairments observed after a focal lesion to the medial and anterior regions of the thalami was similar to that observed after medial temporal lobe damage. These similarities included a prevalent deficit for long term anterograde memory, a less consistent deficit in long term retrograde memory and largely spared short-term memory and implicit memory. Finally, Carlesimo et al. (2011) found that the data deriving from a few single case reports supported the hypothesis of a differential memory role of thalamic regions that had connections with different areas of the medial temporal lobe. That is, the MTT/ATN axis is mainly implicated in recollective memory processes, whereas the ventroamgdalofugal pathway/MD axis was associated with the familiarity process.



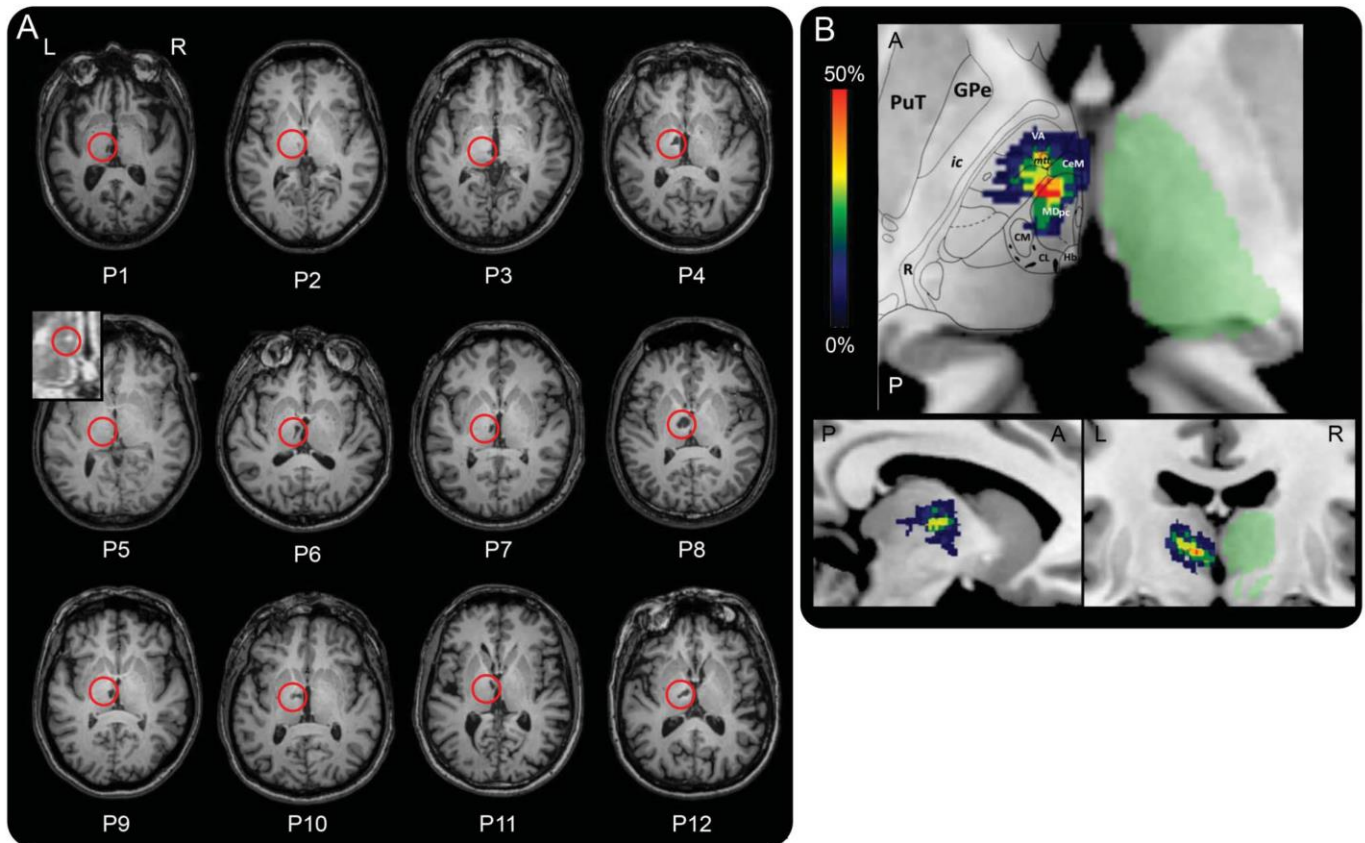


Figure 3.2: Adapted from Danet et al. (2016). Example of a manually segmented and automatically localised thalamic lesion used in their study.

To better understand the role of the MTT, ATN and MD in thalamic amnesia a recent study by Danet et al., (2016) recruited 12 patients with thalamic infarction in the left hemisphere along with 25 matched controls. An advantage of this study over previous work is that the thalamic lesions were manually segmented and automatically localised with a computerised thalamic atlas (figure 3.2). Subsequently, thalamic atlas plates were superimposed onto the summed and overlapped lesions to illustrate lesion distribution across the small tightly packed nuclei of the thalamus. A distinction was made between patients with an intact or damaged MTT. In 11 of the 12 patients the infarct included the mediodorsal nuclei and all had cases had damage to the intra-lamina nuclei. As a group the patients performed worse than controls across verbal memory tasks, but those cases that included MTT lesions showed a more severe memory impairment than cases where the MTT was spared (Danet et al., 2016). Unexpectedly, only one of the patients in this study was found to have explicit lesions to the ATN, which reinforces the difficulty of finding many cases with ATN damage due to selective stroke. As with previous cases of thalamic infarction, this study suggests that lesions to the MTT rather than the MD result in memory impairment.



Although cases of thalamic infarction can result from relatively focal lesions the close anatomical proximity of nuclei and fibre tracts of the thalamus means that lesions involving the MTT invariably extend into adjacent structures, particularly the ATN and MD. This point is best illustrated by the case of the most localized MTT infarct in the literature. Carlesimo et al. (2007) described patient GP whom the authors determined to have localized bilateral damage to the MTT. Patient GP presented with a neurophysiological profile typical of an amnesic syndrome that is, impaired recall of both verbal and visual spatial information with partially spared recognition memory, and normal short-term memory and semantic memory. Figure 3.3, shows the lesion reconstruction and MRI images presented by the Carlesimo et al. (2007). Despite the authors' assessment, they also mention that the infarction likely includes structures adjacent to the MTT, such as the intralaminar nuclei, mammillotegmental tract and ventral anterior thalamus; it may also have included mediodorsal damage, which was not reported. Such evidence reiterates the value of careful experimental animal studies to establish the influence of localized MTT injury.

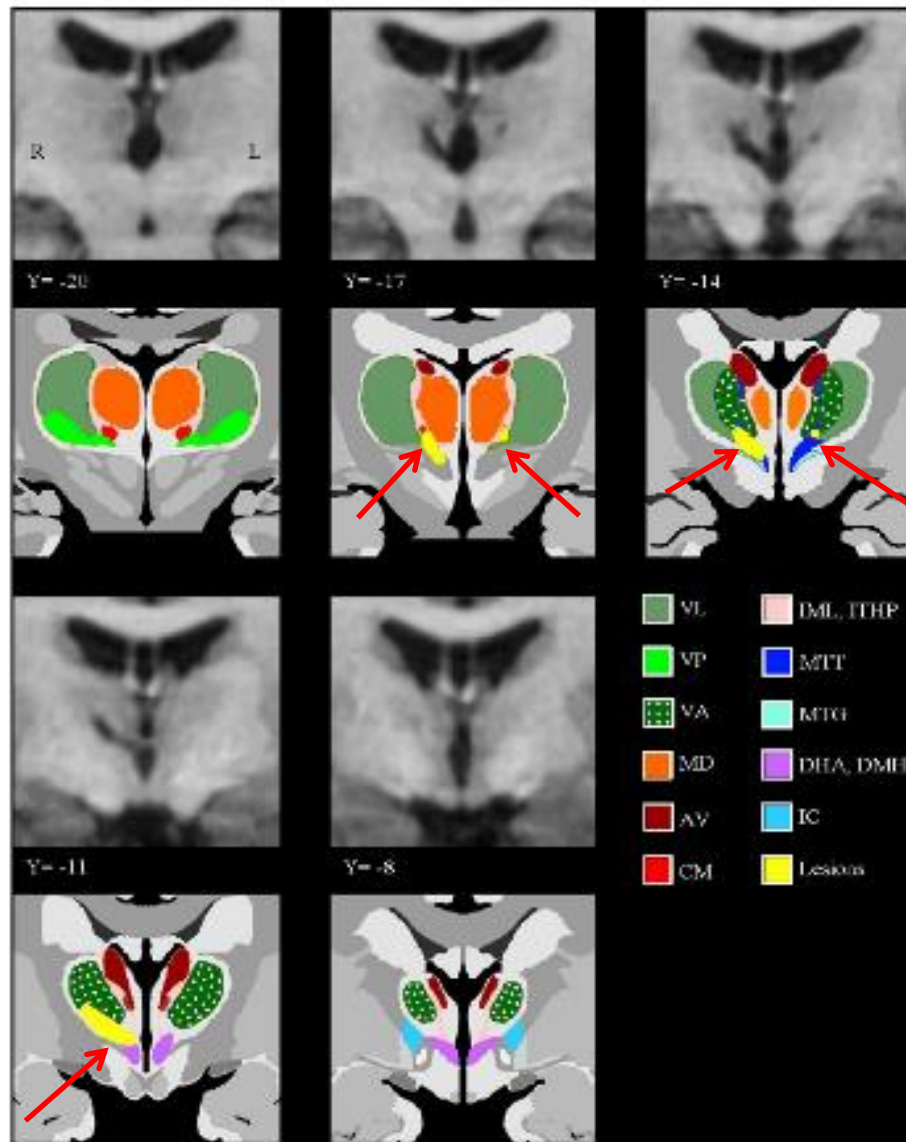


Figure 3.3: Adapted from Carlesimo et al., (2007). Example of a highly localized thalamic lesion from the clinical literature. The images depict five T1-weighted coronal slices (1mm thick) through the thalamus (top row and third row down) with corresponding atlas plates with the lesion superimposed over top in yellow. VL = ventrolateral thalamic nuclei; VP = ventroposterior thalamic nuclei; VA = ventroanterior thalamic nuclei; MD = mediodorsal thalamic nuclei; AV = anteroventral thalamic nuclei; CM = centromedian thalamic nuclei; IML = internal medullary lamina of thalamus; ITHP = inferior thalamic peduncle; MTT = mammillothalamic tract; MTG = mammillotegmental tract; DHA = dorsal hypothalamic area; DMH = dorsomedial hypothalamic nucleus; IC = internal capsule.

### 3.2 Korsakoffs Amnesia

Unlike thalamic stroke, damage to the ATN and not MB or MTT appears to be the critical point of difference between alcoholics with the amnesic Korsakoff syndrome (KS) by comparison to alcoholics with Wernicke's encephalopathy without clinical amnesia (Harding et al, 2000). The Korsakoff syndrome is characterised by a chronic and striking loss of

everyday memory and has long been associated with pathology in the diencephalon (Sechi & Serra, 2007; Kopelman, 2014). More specifically, patients with Korsakoff's typically present with severe anterograde amnesia and are unable to remember events even within the last half hour, but retain implicit learning as they are still able to learn new motor skills and develop conditioned reactions to stimuli. These patients' amnesia therefore closely resembles that of patients with medial temporal lobe injury (Squire & Zola-Morgan, 2011). Korsakoff's amnesia typically results in more profound memory impairments than is found in thalamic infarction cases (Harding et al., 2000; Van der Werf et al., 2003; Carlesimo et al., 2011; Kopelman, 2014). Kopelman's (2014) recent review suggested that both amnesias produce recollection deficits, but patients with Korsakoff's amnesia also invariably have deficits in recognition and temporal context memory as well as retrograde amnesia, the latter of which is only observed in approximately one third of thalamic stroke cases (see table 3.1). Distinctions between these two neurological conditions, but the fact that KS patients generally have neuropathology outside the diencephalon, highlights the value of determining the relative contributions of the MB/MTL and the ATN to memory processes.

**Table 3.1: Comparison of the major neuropsychological (red boxes) and neuroimaging findings between Korsakoff patients and thalamic infarct patients (from Kopelman, 2014).**

<i>Neuropsychology</i>	Korsakoff patients	Thalamic infarct patients
Primary/short-term memory Traditional measures 'Working memory' measures	Intact All components impaired but no worse than non-Korsakoff alcoholics	Intact Not tested
Anterograde episodic memory Recall/recollection Recognition/familiarity Context memory	Severely impaired Impaired, but can be compensated by prolonged exposure times Temporal context memory always affected; spatial more variable	Severely impaired Spared in some studies; impaired in others Recollection deficit implies impairment, but less specifically studied than in Korsakoff's
Retrograde amnesia	Invariably present and usually prolonged (20–25 years) with a temporal gradient	Present in approximately one-third of patients, sometimes 'extensive', sometimes brief (1–10 years)
Confabulation	Seen in acute confusional phase (Wernicke), but spontaneous confabulation in chronic phase only if extensive frontal or ventro-medial frontal damage. Mechanism controversial	Seldom if ever reported
<i>Neuroimaging</i>		
MTL		
Principal findings	Atrophy in thalami, mammillary bodies and frontal cortex	Critical lesions have been reported in the anterior nuclei, mammillo-thalamic tract and medio dorsal nuclei. May be unilateral or bilateral
Associated findings	Atrophy in hippocampi (varying severity), amygdala, and cerebellar vermis	
PET	Reduced metabolism in the thalami, mammillary bodies, basal forebrain/corpus callosum, frontal cortex, retrosplenium/precuneus. Also implicated: middle cingulate gyrus, superior frontal gyrus, temporal and occipital cortex	Few studies: thalamic and retrosplenial hypometabolism implicated. Widespread unilateral cortical hypometabolism has also been reported

In early KS cases it was observed that the mammillary bodies were atrophied in individuals with probable Korsakoff's syndrome and this discovery has subsequently been confirmed in multiple neuropathological studies (Kopelman 2015). Along with MB changes there is often neural loss, gliosis, and micro-hemorrhages, in the MD, paraventricular and peri-aqueductal grey matter (Sechi & Serra, 2007; Kopelman, 2014). This distributed pathology in the diencephalon found in both Wernicke's encephalopathy and Korsakoff's syndrome has made it difficult to determine the critical site of pathology causing the anterograde amnesia in Korsakoff's, and highlight the necessity for appropriate control groups.

Korsakoff's syndrome is often, but not always, preceded by Wernicke's encephalopathy (WE), which is the initial acute neuropsychiatric reaction to thiamine (or vitamin B1) deficiency that is characterised by nystagmus (involuntary eye movement) and ophthalmoplegia (paralysis of extraocular muscles), mental status changes and unsteadiness of stance and gait (Sechi & Serra, 2007, Kopelman et al., 2009). Because the WE phase may not occur or resolve quickly, Korsakoff pathology is now being found more commonly in alcoholics at post mortem than it is in life and is occurring more commonly in younger individuals due to recent changes in drinking habits (Kopelman et al, 2009). Around 80% of those who survive Wernicke's encephalopathy develop KS which is defined as a disproportionate deficit in memory relative to other cognitive features, due usually to thiamine deficiency. Thiamine is an essential co-enzyme for intermediate carbohydrate metabolism, lipid metabolism and production of amino acids and glucose-derived neurotransmitters such as GABA (Sechi & Serra, 2007). Wernicke's encephalopathy is most prevalent in patients who abuse alcohol, but also occurs in various illnesses where the absorption of nutrients has been compromised such as AIDs, anorexia nervosa (purging) and peptic ulcers (Sechi & Serra, 2007). Brain lesions are often restricted to selective and vulnerable areas with a high thiamine turnover and can occur in as little as 2-3 weeks which correlates with the time taken to deplete the body's stores of thiamine as these are only sufficient for up to 18 days (Sechi & Serra, 2007).

### 3.3. Neuropathological studies of KS

An early study by Victor et al. (1971) suggested that pathology within the mediodorsal thalamus was the critical point of difference between WE and KS. More specifically, they

reported that all 24 patients in their study with lesions in the mediodorsal thalamic nucleus had exhibited a persisting memory disorder. By contrast, five patients with MB lesions but spared mediodorsal nuclei only experienced transient WE without developing amnesia (Victor et al, 1971). However, this study was later criticized for lacking detailed neuropsychological evidence to corroborate their neuropathological observations.

Two later studies by Mair et al. (1979) and Mayes et al. (1988) both employed more rigorous and detailed neuropsychological assessments which were combined with extensive examinations of postmortem tissue, in two patients each. In contrast to Victor et al's. (1971) findings, both Mair et al. (1979) and Mayes et al. (1988) concluded that pathology within the mammillary bodies, the mammillothalamic tract and the anterior thalamus had a critical role in the genesis of the anterograde amnesia present in these four patients.

A particularly informative study by Harding et al. (2000) has provided the strongest clinical evidence implicating the ATN pathology in the development of an amnesic syndrome. Improving on previous studies, these authors further delineated the critical site for anterograde amnesia by including appropriate control groups and using unbiased stereology to estimate cell counts in each previously implicated structure. Harding et al. (2000) compared five WE-only patients with eight WE/KS patients as well as alcoholic and non-alcoholic controls. In line with previous findings, neurodegeneration in the mammillary bodies and mediodorsal thalamic nuclei was substantial in both WE and WE/KS patients (figures 3.4 and 3.5), but substantial neuronal loss in the anterior thalamic nuclei (often referred to as the anterior principal nucleus in humans) was only consistently found in WE/KS patients (figure 3.6).

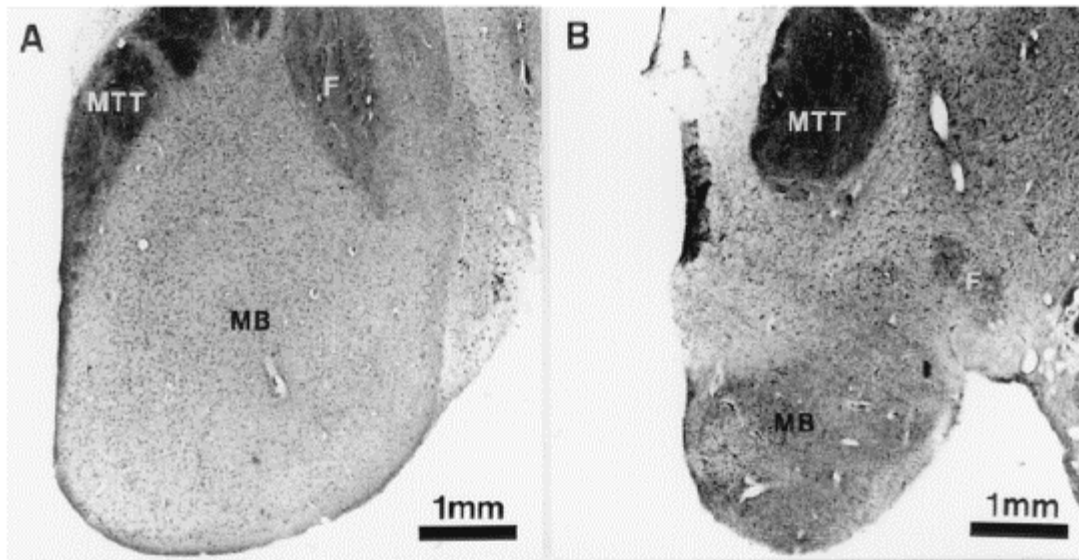


Figure 3.4: Adapted from Harding et al. (2000) photomicrographs comparing the MB of a health control (A) to those of an alcoholic with chronic WE (B). Of note is the shrunk darkened appearance of the MB in the WE patient.

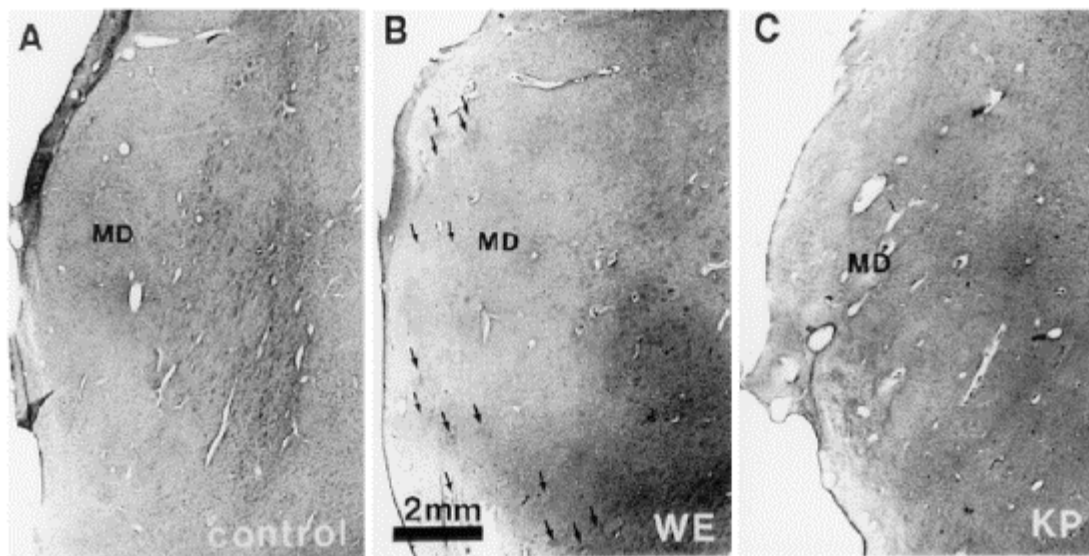


Figure 3.5: Adapted from Harding et al. (2000) photomicrographs of the right mediodorsal (MD) nucleus of the thalamus from (A) a health control, (B) a patient with WE and (C) a patient with KS. Similar pathology including petechial hemorrhages (arrows) present in both B and C.

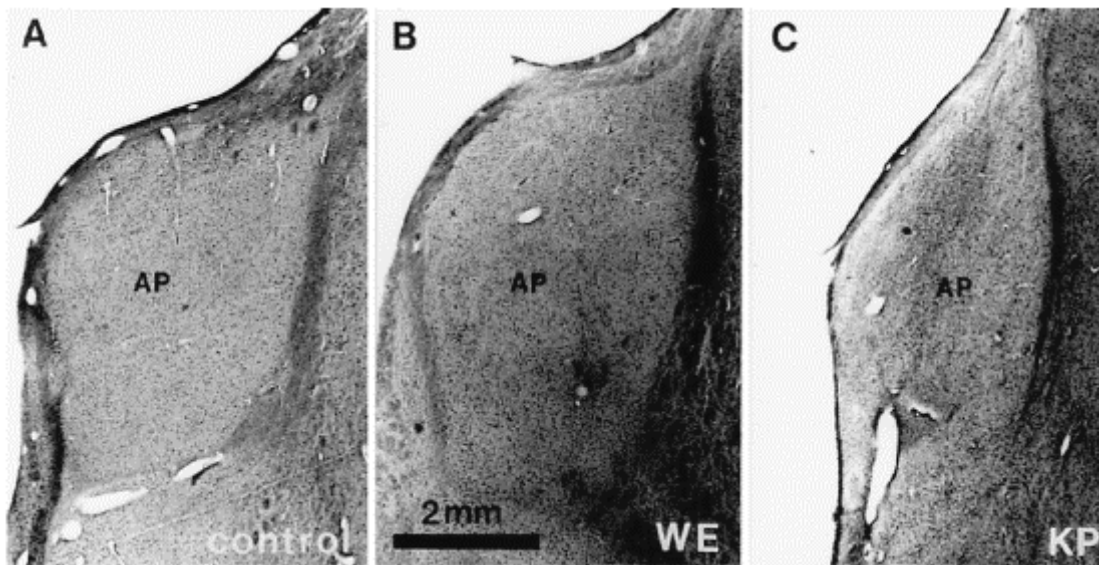


Figure 3.6: Adapted from Harding et al. (2000) photomicrographs of the right anterior principle nucleus (AP aka ATN) in a healthy control (A), a patient with WE (B) and a patient with KS (C). Notice the considerable atrophy in the KS patient (C) along with blood vessel, myelin and parenchymal abnormalities.

### 3.4. Neural imaging studies in KS

Consistent with neuropathological findings neural imaging studies have provided additional evidence for the involvement of the thalamus and mammillary bodies in the anterograde amnesia associated with KS (Kopelman, 2014). A study by Pitel et al. (2012) compared gray and white matter volumes in alcoholics with and without KS. Their results revealed considerable similarities in the pattern of grey and white matter damage between the two groups. However, a few key regions, including the medial thalami, mammillary bodies and corpus callosum were more severely damaged in KS patients than the alcoholic control group (Pitel et al., 2012). Similarly, a common finding in 18-fluoro-deoxy-glucose metabolism on positron emission tomography (PET), a functional measure, is reduced glucose uptake in the thalami bilaterally, the mammillary bodies, and the basal forebrain/orbito-frontal cortex (Reed et al., 2003; Pitel et al., 2009). Current imaging techniques are improving, but they still lack sufficient resolution to accurately dissociate the small parcellated nuclei and fibre pathways within the thalamus with sufficient detail for specific tests of neuroanatomical findings in-vivo.



Taking a different approach, Kim et al. (2009), provided additional evidence for the role of the MB, MTT and ATN in memory impairments associated with Wernicke's encephalopathy. Kim et al. (2009) compared 7 chronic alcoholics with Wernicke's encephalopathy and recovering memory function, with 14 alcoholics without Wernicke's encephalopathy and 14 healthy controls. The participants underwent functional connectivity fMRI scans, as well as verbal and non-verbal memory tests. Resting state functional connectivity between the ATN and the MB was estimated during a five minute viewing task. Kim et al. (2009) found the memory function in patients recovering from Wernicke's encephalopathy paralleled the level of resting state functional connectivity between the MB and ATN.

### 3.5. Neurodegenerative disease

Other disorders that affect the thalamus include multiple sclerosis, the Lewy body disease spectrum, and Alzheimer's dementia. MRI studies of multiple sclerosis have found that a reduction in thalamic volume was the strongest predictor of overall cognitive functioning (Houtchens et al., 2007), and patients with the most severe cognitive impairments had the lowest thalamic volumes (Schoonheim et al., 2015). In addition, a recent multicenter study reported that pronounced atrophy in the anterior thalamic regions and abnormal diffusion tensor MRI indices of all cortico-thalamic tracts differentiated cognitively impaired and cognitively intact MS patients (Bisecco et al., 2015). However, atrophy in the CA1 subregion of the hippocampus has also been shown to correlate with impaired encoding and retrieval in MS patients (Geurts et al., 2007; Sicotte et al., 2008) suggesting widespread pathology may be responsible for cognitive decline. Evidence from autopsy examinations of patients with clinically diagnosed and neuropathologically confirmed Parkinson's disease (PD) were found to have Lewy bodies, the hallmark of Parkinson's pathology, within many regions of the thalamus, but especially in the limbic nuclei (Rub et al, 2002). In a more recent multimodal (MRI, diffusion tensor, proton MR) imaging study, Delli Pizzi et al. (2015) examined the involvement of the thalamus in the fluctuating cognition and attention often observed in dementia with Lewy bodies and contrasted these patients to patients with Alzheimer's disease (AD) (Delli Pizzi et al., 2015). Severe episodic memory impairment is a cardinal feature of AD, whereas dementia associated with Lewy bodies tends to manifest relatively greater attentional, visual and executive impairments than AD (Tröster et al, 2008). Delli Pizzi et al.



(2015) found that patients with dementia with Lewy bodies had bilateral damage to the thalamic regions that project to the prefrontal cortex, whereas AD patients showed bilateral alterations within the anterior thalamic regions and pulvinar which project more to the limbic cortex and medial temporal lobe. This study supports the role of an extended hippocampal network in the episodic memory deficits associated with AD.

In accordance with the aforementioned findings a recent review by John Aggleton and his colleagues proposed that it is time to make a conceptual shift towards the inclusion of regions beyond the medial temporal lobe in the memory loss associated with neurodegenerative disorders, particularly Alzheimer's disease (Aggleton et al., 2016). AD-related memory impairment has primarily focused on medial temporal lobe (MTL) pathology because of the clinical and experimental literature since the case of H.M. and the MTL has been emphasized as a site of severe, early neurofibrillary tangle pathology in AD. However, both anterior thalamic and hippocampal changes have been found to occur relatively early in the progression of the disease (Braak & Braak 1991a,b; Delacourte et al., 1999). Imaging studies have provided additional evidence for thalamic involvement in AD (Aggleton et al., 2016). For example, de Jong et al., (2008) found that in addition to the expected decrease in global grey matter and hippocampal volumes, profound atrophy was observed in the putamen and thalamus in patients diagnosed with probable AD. Moreover, it was found that decreased volumes in each of these four regions correlated linearly with impaired global cognitive performance. An important early study, by Nestor et al. (2005), compared AD patients to patients with semantic dementia, a condition that affects semantic memory relatively more than episodic memory. While MRI imaging suggested similar reduction in MTL integrity, Positron emission tomography revealed that the episodic memory impairment in AD was associated with metabolic dysfunction of an integrated network involving the medial temporal lobe, mammillary body region, mediodorsal thalamus and posterior cingulate. By contrast, the diencephalic network was relatively intact in the semantic dementia patients, who instead had hypoactivation within the rostral temporal lobes (Nestor et al., 2005).

### 3.6. Developmental Amnesia

Recent evidence has pointed to a previously overlooked role for the mammillary bodies and anterior portion of the thalamus in developmental amnesia (Dzieciol et al., 2017).

Developmental amnesia is associated with a hypoxia-ischemic event typically occurring in early life. Previous research has focused on the role of the hippocampus because it is especially vulnerable to hypoxic episodes, although damage also occurs in the thalamus and

mammillary bodies (Thayyil et al., 2010; Jokura & Naito, 2008; Schmidt-Kastner & Freund, 1991). Dzieciol et al. (2017) performed MRI on 18 patients with developmental amnesia all presenting with deficits in episodic memory recall, despite relatively well preserved intelligence, semantic memory and recognition memory. In addition to the expected hippocampal atrophy the MB were damaged to such an extent that they were not identifiable in two thirds of the cases, and the remaining 6 cases also had significant MB reduction. Moreover, Dzieciol et al. (2017) found that the volume of the anterior two-thirds of the thalamus was highly correlated with visual memory performance in these patients, even though the posterior segment of thalamus was also similarly reduced in size. Such evidence reinforces the involvement of the diencephalon and a need to focus on network wide changes associated with poor episodic memory beyond the hippocampus.

### 3.7. Traumatic brain injury

There are many case studies examining memory function after traumatic brain injury to the temporal lobes and other cortical sites. By contrast, reports of anterograde amnesia resulting from traumatic brain insults to the diencephalon are rare. Traumatic brain injury provides an important perspective as an exact time course for the insult can be established. Two cases are often cited in the literature (Squire et al, 1989; and Dusior et al, 1990). While these cases are interesting to consider both are confounded because damage to multiple diencephalic sites occurred as a result of the penetrating injuries.

Squire et al. (1989) described patient N.A. who sustained a penetrating brain injury when a miniature fencing foil was accidentally thrust up his right nostril. A series of MRI studies conducted more than 20 years after the insult revealed three major areas of brain damage (Squire et al., 1989). A large lesion was observed in the left thalamus that interrupted the intralaminar and mediodorsal nuclei, but also likely transected both the MTT and the post-commissural fornix. The posterior hypothalamus was also markedly disrupted and the mammillary bodies appeared to be missing bilaterally. There was also damage to the anterior temporal lobe which extended to the amygdaloid complex. Patient N.A. presented with a verbal memory impairment that was considered to be unusually pure, differentiating him from patients with Korsakoffs syndrome. Additionally, unlike patients with Korsakoff syndrome, he showed good insight into his memory problem, accurately predicting his subsequent performance on a recognition memory test. Although Squire et al. (1989)

attributed the memory dysfunction to the internal medullary laminae and mammillothalamic tract damage, many structures relevant to memory were disrupted by the trajectory of the penetrating object making attribution of the amnesic syndrome to a single structure difficult.

The second instance of a penetration injury resulting in severe memory impairment concerned patient B.J. (Dusior et al, 1990). Patient B.J. received accidental brain damage when a snooker cue entered his left nostril into the basal regions of the brain. In the initial period after his injury his memory disorder had the clinical features of a dense amnesic syndrome with both retrograde and anterograde amnesia. However, formal memory testing 21 months after the insult showed marked verbal memory impairment, but relatively intact non-verbal memory and the retrograde amnesia had regressed to mainly affect the period 6 months before the injury. MRI showed bilateral damage to the MB. Importantly there was no damage to the body of the thalamus implicating MB injury as the primary cause of this memory loss.

More detailed evidence for the involvement of the MB in episodic memory has come from damage secondary to removal of diencephalic colloid cysts in humans. Aggleton and colleagues (Aggleton et al, 2010; Aggleton, 2008) have long suggested that an important consideration in understanding the functional relationship between temporal lobe amnesia and diencephalic amnesia depends on determining the role of the fornix, the major interlinking tract (see figure). A study by Tsivilis et al. (2008) related fornix volume with memory impairment following the removal of colloid cysts. A colloid cyst is a benign tumor that develops in the third ventricle adjacent to the fornix. These cysts are normally surgically removed, but this often causes substantial damage to the fornix. Fornix status was assessed directly by fornix volume. MB volume was also assessed as atrophy is observed in this structure following fornix damage. Fornix volume was not consistently correlated with memory performance. By contrast, Tsivilis et al. (2008) found that MB volume significantly predicted the recall of episodic information and correlated significantly with 13 of 14 recall memory tests and 7 of 8 memory indices. Furthermore patients with the smallest MB volume performed significantly worse on tests of recall than those with the largest MB volumes. No correlations were observed between MB volume and recognition memory performance.

Despite the considerable evidence implicating MTT damage in clinically defined cases of the amnesic syndrome not all reports of MTT damage result in a memory deficit. For example, Duprez, et al. (2005) stereotactically implanted stimulation electrodes through the

MTT to reach the MB in three patients to treat chronic refractory epilepsy. Each of the stimulation electrodes had four contacts, three that were located within the MTT and one located within the MB. They reported that, none of the three patients experienced any clinically significant memory deficit either immediately after the surgical implantation or during stimulation. Such implants passed through or adjacent to the ventral aspects of the MTT but appear to have caused significant neural injury to the MTT (figures 3.7 & 3.8). Although not reported, therapeutic deep brain stimulation for refractory epilepsy typically utilizes high frequency stimulation (100 + Hz), which would be expected to result in local inactivation and thus memory deficits. The authors suggest that comprehensive cognitive testing was performed throughout, all of which failed to reveal any early or delayed mental decline after implantation. Unfortunately, Duprez et al. (2005) did not give any details regarding which cognitive tests were administered, how their patients scored relative to matched controls or at which specific time points testing was conducted. Duprez et al. (2005) suggest perhaps the lack of memory impairment can be attributed to the alternative pathway of the intact direct route from the hippocampus via the fornix to the ATN. Their proposal suggests that MB or MTT neuropathology, even if bilateral, may not be as disruptive as ATN neuropathology, a perspective that resonates with some key findings in the KS literature and the possibility that damage additional to the MTT may contribute to the severity of amnesia after thalamic stroke and developmental hypoxia-ischemic encephalopathy.

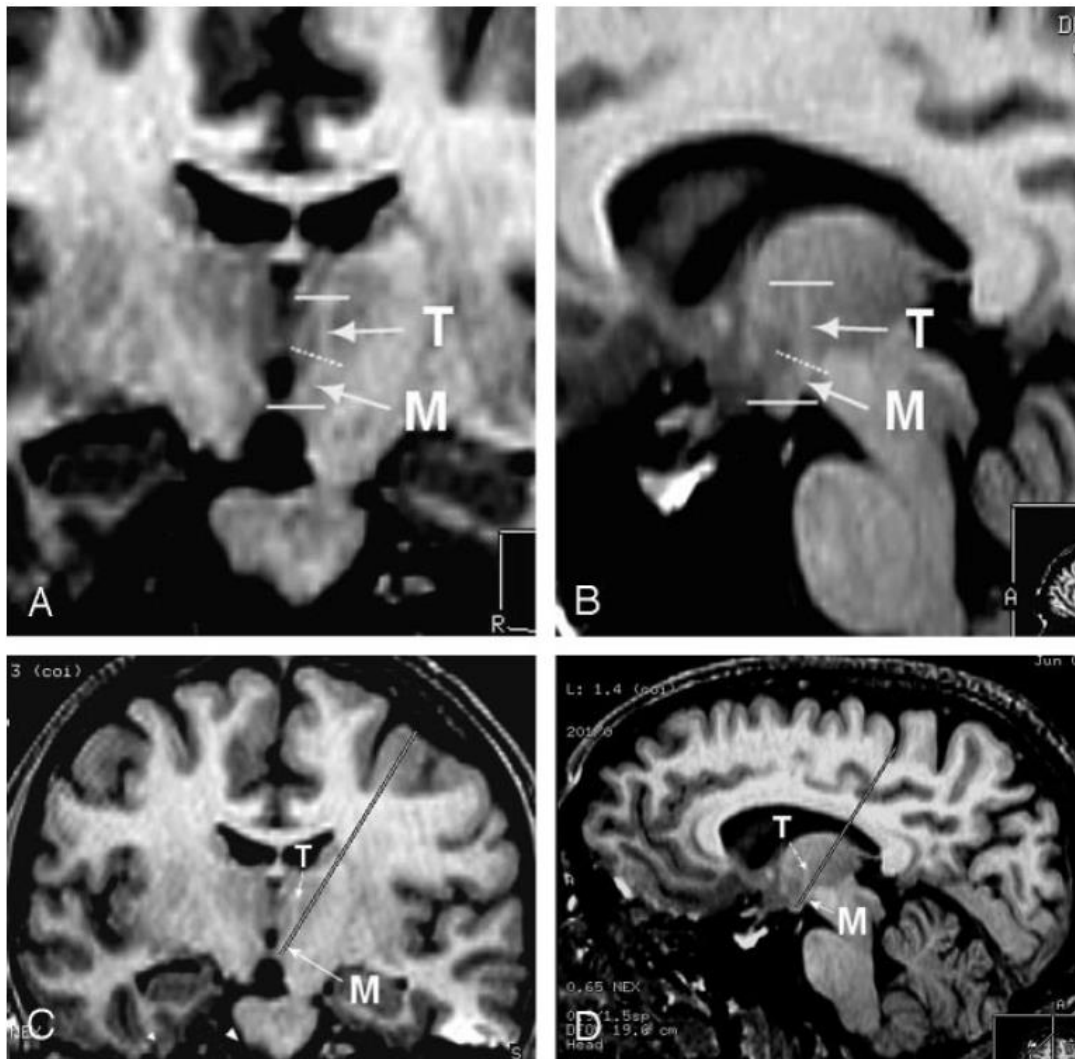


Figure 3.7: Adapted from Duprez et al. (2005). T1 weighed images indicating the location of the MTT (T) and MB (M) in both a control patient (A & B) and the trajectory of the bilaterally implanted stimulation electrodes in patients with refractory epilepsy (C&D). Notice the electrode follows along the ventral aspect of the MTT down into the MB.

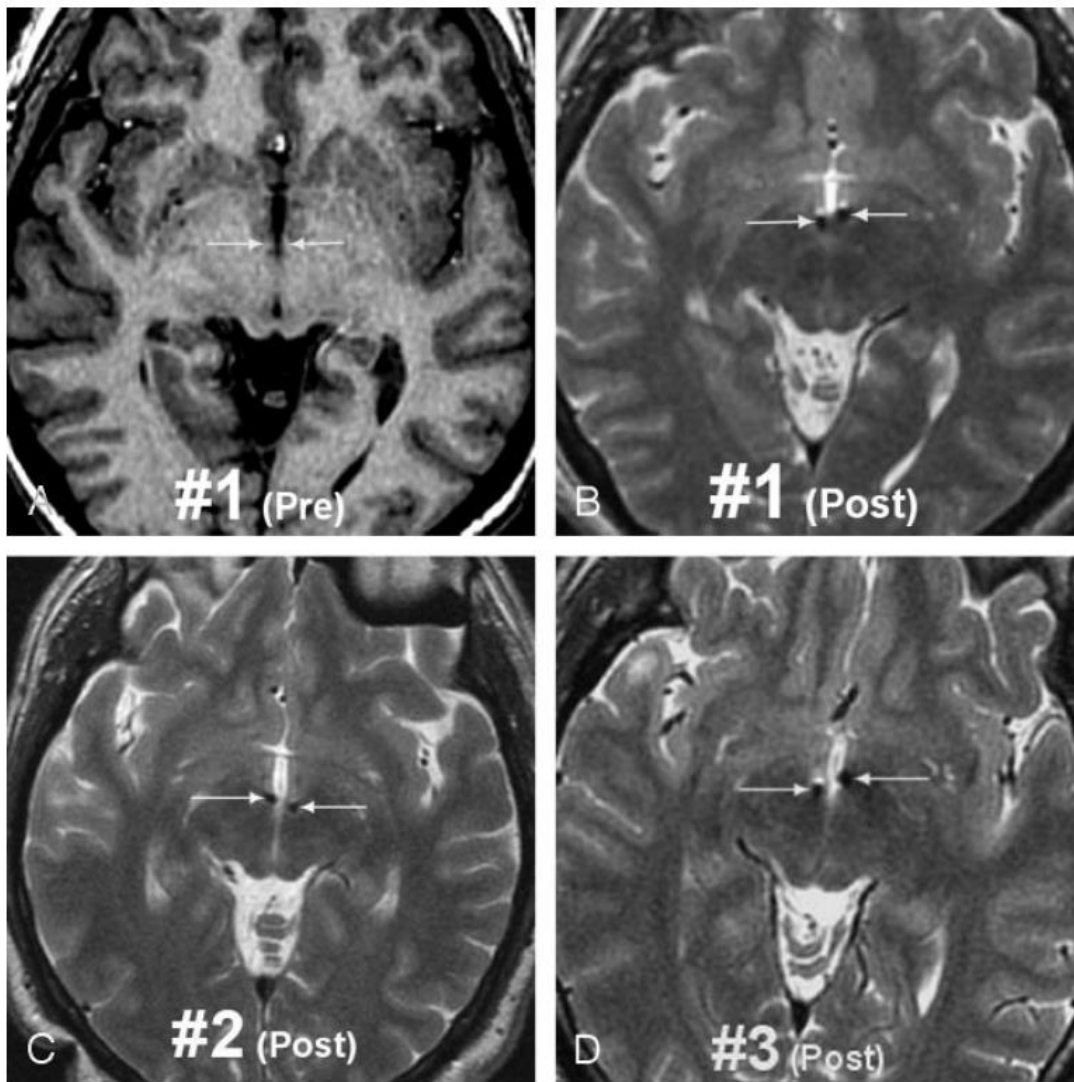


Figure 3.8: Adapted from Duprez et al. (2005) T1 weighted MRI image of MTT (arrows) preoperatively (A). T2 weighted FSE images showing the location of the bilateral stimulation electrodes (arrows) in each of the three patients postoperatively (B,C & D).

### 3.8. Concluding remarks

The strongest clinical evidence for MTT-related memory impairments has come from cases of localized thalamic stroke, with less consistent evidence for additional thalamic regions including the ATN. Damage to the ATN appears to be the critical point of difference between the amnesic Korsakoff syndrome and patients with Wernicke's encephalopathy, although MB pathology is also consistently reported across studies (Kopelman, 2014; Carlesimo et al., 2011; Harding et al., 2000). Neurodegenerative disease, developmental amnesia and traumatic brain injury have provided additional evidence that damage to the MTT, MB and anterior thalamic regions contribute to the severe episodic memory deficits observed in these

conditions. Clearly both the MTT and ATN are critical sites of pathology for the development of anterograde amnesia. However, differences in the severity of and extent of memory impairments associated with Korsakoff's amnesia and thalamic stroke hint that damage to these two structures might result in different deficit profiles or severity of impairment. However, the frequent additional brain injury apparent in even the most localized of clinical cases precludes firm conclusions regarding each structures specific contribution to memory. Animal lesion models have helped address the problem of lesion specificity in clinical studies and allow precise control over critical outcome measures. To date, however, an explicit comparison of ATN and MTT lesions has not been conducted. The following chapter reviews the evidence from animal lesion studies with particular focus on the MTT and ATN.

## Chapter 4

# Animal models of diencephalic amnesia

This chapter reviews experimental findings from animal lesion studies with an emphasis on the mammillothalamic tract (MTT), the anterior thalamic nuclei (ATN) and memory. As discussed in the chapter on diencephalic amnesia, human pathology invariably includes damage to multiple brain regions, and even in the most localised example extended to adjacent regions (Carlesimo et al., 2007). Animal models provide the advantage of detailed post-mortem examination, the ability to produce relatively precise localised lesions, and the direct comparison of lesioned animals to sham surgery controls in pre and post-surgery testing. One major caveat, of course, is the degree to which memory tasks used in animals are analogous to episodic memory in humans, which is discussed in the next section. Following this, it is clear that there is a relatively limited literature on the effects of MTT lesions, which are the focal point for radiofrequency lesions in the present study. A key issue is the comparison of MTT lesions with the more extensive literature regarding ATN lesion effects. A case is then made that a direct comparison between MTT and ATN lesions is necessary to test two competing perspectives regarding the relative influence of these structures in diencephalic amnesia. The first perspective suggests that the ATN is a critical node within an extended hippocampal system with an important role in multiple subcortical-cortical interactions. An alternate view emphasises the importance of the unique brainstem input from the tegmental nuclei of Gudden as the primary influence on the ATN, via the MB and MTT, and hence the extended memory network. That is, the question is whether the ATN have an influence on cortical (including hippocampal) structures of the extended system beyond its influence from the MB and the information conveyed in the MTT.

The subsequent section will explore the effects of lesions to the MB in which the cell bodies of the MTT axons are located, VTg lesions and their influence via the MB/MTT, and the effects of post-commissural Fx lesions which provides an indirect hippocampal influence on the ATN via the MB/MTT. This review will focus on rodents, but studies using non-human primates have been included where relevant. Lastly, studies have reported “covert pathology” in distal brain regions such as the hippocampus and retrosplenial cortex as a direct



result of MTT and ATN lesions; this work will also be summarised. These latter studies provide direct evidence that lesions within the diencephalon can have a direct influence on the function of the wider neural circuit. This idea will be expanded on in the subsequent chapter in which the role of rhythmic oscillatory activity will be explored in the long-range interactions occurring between neural structures, including the extended hippocampal circuit. A proposal is made that memory deficits following lesions to the diencephalon may in part relate to disrupted electrophysiological communication between distal regions such as the retrosplenial cortex, hippocampus and prefrontal cortex, beyond the static influence of lesions in terms of distal structural diaschisis.

#### 4.1. Examining episodic like memory in animals

One obstacle faced by researchers wanting to model memory processes in animals is how to capture something akin to human episodic memory. Episodic memory refers to the conscious recollection of a personal experience that contains information of the content of what has happened as well as their spatial and temporal context (Pause et al., 2013). The recollection involved in episodic memory has been proposed to imply a first-person subjectivity that has been termed *autonoetic consciousness* (Tulving, 2001). For this reason episodic memory as we know it is probably unique to humans, but its fundamental characteristics and general neuroanatomy may be universal among mammals. Considerable research using animal models has been conducted over the last two decades to determine the validity of this premise. Evidence from these studies has shown that various animal species can also show behavioural manifestations of different aspects of episodic memory such as the capacity to remember information regarding what happened, and where these events took place and when the items in the event occurred (Binder et al. 2015).

A now famous experiment by Clayton and Dickinson (1998) provided the first evidence of what-when and where memory in non-humans. These the authors used scrub jays as experimental subjects. These birds are known for storing food in different locations for later consumption. Two types of food were used in this experiment that enable a direct comparison of spatial and temporal context: wax worms which are preferred by the bird but spoil readily, and peanuts which are less preferred but stay fresh for longer time periods. The birds were allowed to cache each food-type at visuospatially distinct places. In one condition, the birds were allowed to cache peanuts and then 5 days later allowed to cache worms in a separate place, followed by a choice test 4 hr when the birds chose the place that should have the (preferred) worms and not the place that should have the peanuts. In the second condition,

the birds were first allowed to cache worms and then 5 days later allowed to cache peanuts followed by the choice test 4 hr later between the two places where worms and peanuts might be found, at which point the birds chose the place for peanuts rather than the place for worms. The authors suggested that this pattern of results indicated that the birds remembered what type of food they had cached, as well as when and where.

In rodents, attempts to extract episodic-like memory have combined multiple one-trial object exploration paradigms including modified novel object recognition, object place and temporal order memory (Dere et al. 2005; Kart-Teke et al., 2006). In the Dere et al. (2005) modified object task procedure, task mice were given two sample phases and one test phase. In the first sample phase the animal is placed in a large arena with four identical novel objects and allowed to explore. After a 50 minute delay, the animals are returned to the arena for a second sample phase in which a different set of four identical objects are present. After an additional 50 minute delay the animals receive the test phase, in which two copies of the objects from sample 1 (old familiar objects) and two objects from sample 2 (recent familiar objects) are presented together. Importantly, one old object is spatially displaced and presented together with both familiar objects which remain at familiar locations (Dere et al., 2005). Intact animals spend more time exploring the two old familiar objects, reflecting memory for what and when, whilst also directing more exploration towards the spatially displaced old familiar object relative to the stationary old familiar object, which is taken as evidence for what and where (Binder et al., 2015).

The most extensively used tasks in rodents, however, are working memory tasks in which the animal has to keep changing what it should do in light of what it has just done (Morris, 2001). Examples of this type of task include the radial arm maze, delayed non-matching to place in the T-maze/cross maze and delayed matching to place in the water maze (Olton et al., 1979; Rawlins 1985; Morris 1983). These tasks all rely on spatial memory, particularly allocentric spatial memory. While such tasks focus aspects of “where” and “when”, given their working memory nature, but fail to accommodate item memory (“what”) they are useful in that hippocampal system lesions invariably impair performance on these tasks.

The T-maze relies on rodent’s natural tendency to alternate spatial responses, and is comprised of two parts, a ‘sample’ and a ‘choice’ run. During the ‘sample’ run one arm extending from a central stem is blocked directing the animal down the unblocked arm for

reward. On the subsequent ‘choice’ run the previously blocked arm is now open and the rat must now enter this arm to gain reward (Aggleton & Nelson, 2015). The radial arm maze (RAM) consists of a central hub (usually octagonal) with eight (or more) arms extending off it in all directions. In the standard version of this task, all eight of the arms are baited only once and the optimal strategy is for the animal to visit each arm without revisits, requiring it to remember where it has previously been. The water maze consists of a large circular pool with an escape platform hidden just below the surface of the opaque water. In this task the animal is naturally motivated to escape the water by locating the submerged platform. In the water maze spatial learning is measured by more accurate navigation to the platform location across successive trials and can accommodate working memory versions of the task. In all such tasks, however, non-spatial factors may also influence performance.

Intact animals likely use a combination of egocentric and allocentric strategies to solve spatial memory tasks of this nature (Aggleton & Nelson, 2015). Egocentric strategies involve using intra-maze cues and body-turn information to solve the maze. Allocentric strategies involve combining distal cues to create a spatial representation that is independent of the animal’s current position. The acquisition of allocentric spatial memory has been repeatedly demonstrated to be sensitive to lesions within the extended hippocampal memory system, whereas egocentric acquisition strategies are generally not (Aggleton & Nelson, 2015). The utility of these tasks is twofold, as not only are they readily learnt by intact animals but they require the animal to use external cues to create a spatial representation of the environment and these tasks have been extensively used following lesions to the extended hippocampal system (Aggleton 2008, Aggleton & Nelson 2015). This allows researchers to make comparisons regarding the relative impact of different structures on spatial memory. For this reason the RAM and the water maze were used as the benchmark tests in the current study.

## 4.2. MTT and ATN lesion studies

It is noteworthy that, despite its importance in human diencephalic amnesia, relatively few studies have examined the behavioural impact of MTT lesions in rats. This provides a stark contrast to a well-established ATN literature (tables 4.1 & 4.2), which are generally less emphasised in a clinical context because ischemic stroke seldom produces relatively focused damage in the ATN (Van der Werf et al, 2003). Moreover, the early MTT lesion studies are difficult to interpret because of poor lesion localisation, extensive non-MTT damage, and the use of poorly validated behavioural tasks. For example, Thomas and Gash (1985)

produced extensive lesions to the MTT by electrolysis (figure 4.1a), which will have caused substantial damage to adjacent structures including the nucleus reuniens in many cases (panel D) and probably damaged the supramammillary nuclei in many, both of which have memory relevant functions. By contrast, recent studies by Vann and colleagues and Winter et al., (2011) have made a concerted effort to create localised lesions to the MTT using a radio frequency probe or small electrolytic lesions to allow tighter control over the resulting lesion area (see figure 4.1b). For this reason, emphasis is given to studies in the modern era that began with Vann, Aggleton and Honey (2003), summarised in table 4.1. Looking across both MTT and ATN lesion studies there has been a focus on spatial memory testing, often in the T-maze, radial arm maze and water maze. This is not surprising given that these tasks have been extensively used following hippocampal and fornix lesions. The following section contrasts MTT and ATN related memory deficits across separate studies and provides a case for a direct comparison between these two structures. Such a comparison would provide important insights into two different perspectives for the key site of pathology in diencephalic amnesia. The first perspective emphasises the role of the brainstem inputs into the extended hippocampal system via the MB/MTT and as previously mentioned is supported by cases of thalamic stroke (Carlesimo et al., 2011). An alternate perspective emphasises the role of the ATN as a critical node both relaying and modulating information to and from cortical and subcortical regions (Jankowsky et al., 2013; Aggleton et al., 2016). The latter perspective receives its strongest clinical support from neuropathological evidence in the WE/KS syndrome (Harding et al., 2000).

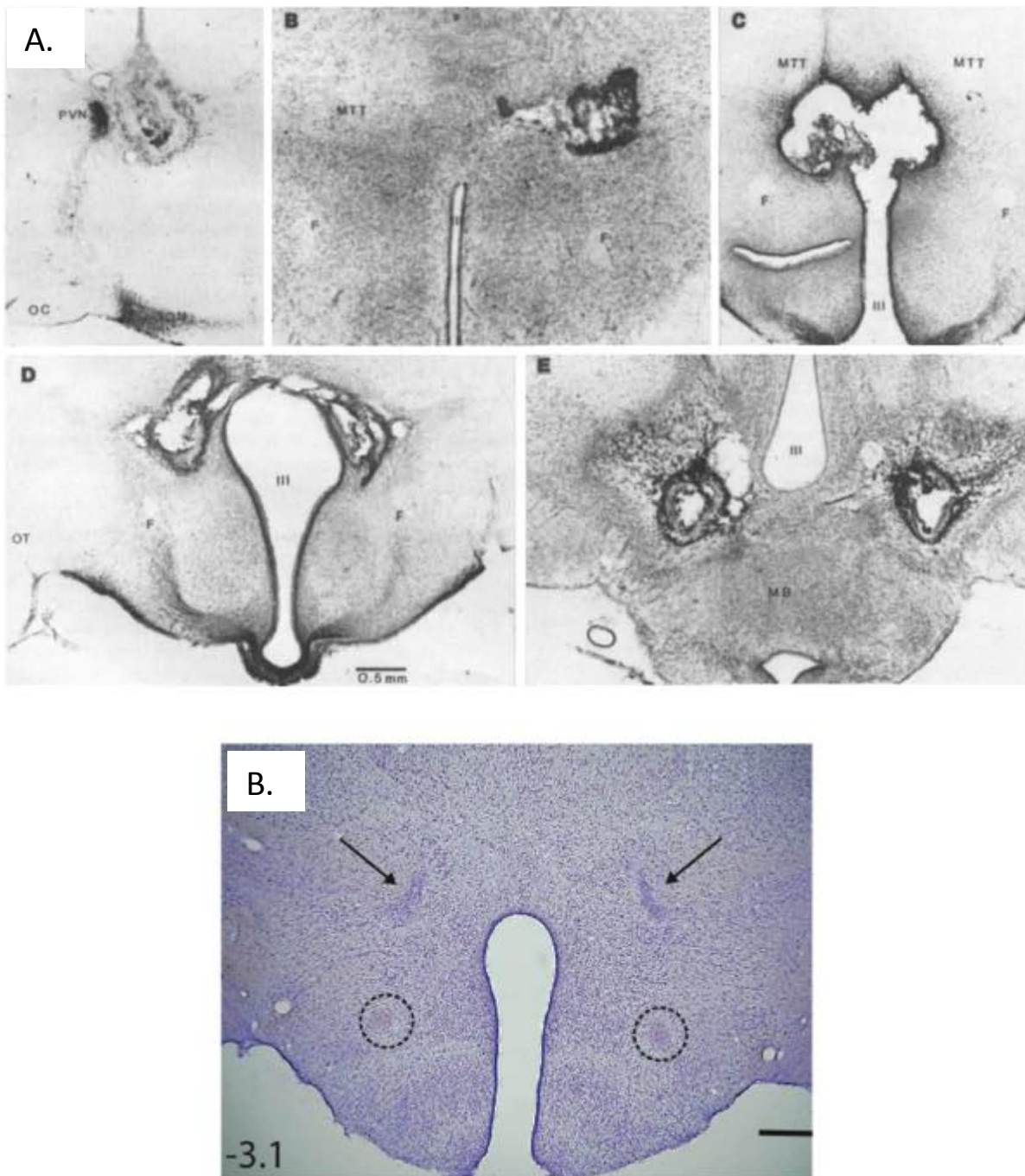


Figure 4.1: Photomicrographs of MTT lesions showing considerable improvement in lesions localisation from an early study (A; adapted from Thomas & Gash, 1985) to a recent study (B; adapted from Vann, 2013).

MTT lesions result in a spatial memory deficit on the standard T-maze task that is in some instances transient (Vann & Aggleton 2003; Vann 2013). For example, Vann (2013) found that MTT lesion deficits only appeared when intra-maze cues were minimised by running the rats on two adjacent mazes or when intra-maze and allocentric cues were also

removed by running the rats in the dark. By contrast, a more recent study, by Frizzarti et al., (2016), found rats with MTT lesions had a persistent deficit in standard T-maze alternation. Unlike MTT lesions, ATN lesions have repeatedly been shown to result in a severe and pervasive impairments in the standard T-maze although some variation in the severity of deficit can be found across studies (Aggleton et al., 1995, 1996, 2009, Loukavenko et al., 2007; Dumont & Aggleton, 2013). In these rats, spatial alternation performance often starts close to chance levels and although improvement is sometimes seen, rats with ATN lesions fail to reach normal levels of accuracy, even if trained prior to lesion surgery (Warburton et al., 1999). These deficits are especially pronounced when the use of egocentric (body turn information) is controlled for by using a t-maze embedded within a cross maze, in which sample and test runs can be run from opposite ends of the maze (Loukavenko et al., 2007, 2016; Aggleton & Nelson, 2015).

Unlike the T-maze, MTT lesions produce a more consistent spatial working memory deficits when tested in the radial arm maze (RAM) (Vann & Aggleton 2003; Vann 2013; Nelson & Vann 2014). In two separate experiments, MTT lesions were found to replicate the deficits found following neurotoxic MB lesions (Vann & Aggleton, 2003) and were also equally impaired to VTg lesions (Vann, 2013) on the standard working memory task in the RAM. The addition of a slight manipulation to the standard RAM task has been shown to profoundly exacerbate the MTT deficit (Vann & Aggleton, 2003; Vann, 2013). In this task, the RAM is rotated by 45 degrees midway through a trial and the remaining rewards moved to retain their relative spatial location. This manipulation places intra and extramaze cues in conflict and is also particularly sensitive to lesions of the retrosplenial cortex (Pothuzian et al., 2009; Vann & Aggleton 2002, 2004). Not surprisingly, ATN lesions also produce severe deficits on the standard RAM task (Sziklas & Petridies, 1999; Mitchell & Dalrymple Alford, 2006; Alexinsky, 2001; Sziklas & Petrides, 2007). Additional modifications to the standard RAM, such as delayed non-matching to sample (Mair et al., 2003), and a version in which the rats must constantly recall the location of baited and un-baited arms throughout training, also show significant deficits in rats with ATN lesions (Byatt & Dalrymple-Alford, 1996; Harland et al., 2014).

MTT lesions also result in impaired spatial working memory in the water maze (Vann & Aggleton, 2003). In the working memory version of the water-maze task the platform location remains stable within a session, but varies between sessions requiring rapid encoding of spatial information. Vann & Aggleton (2003) found rats with MTT lesions had

significantly longer path lengths and escape latencies than sham controls to the platform across 12 days of testing. Illustrating the need for more studies on MTT lesion effects, the only other laboratory to use selective MTT lesions reported that rats with MTT lesions were not impaired relative to shams on spatial working memory in the water maze (Winter et al., 2011). However, this latter study used a simplified version of the working memory task in that rats were only tested for two trials a day for four days and, in particular, the location of the platform relative to the edge of the pool was not changed so that alternate non-spatial strategy might explain this negative finding. ATN lesions have also been shown to severely impair spatial working memory in the water maze and the severity of this deficit was found to increase with the extent of anterior thalamic damage (Van Groen et al., 2002). However, even rats with total ATN lesions may show spatial working memory improvements with extensive training (Van Groen et al., 2002).

The water maze task was, however, first devised to test spatial reference memory, using a fixed escape location, and this classic task provides an interesting point of difference between MTT and ATN lesions. MTT lesions have only been examined on standard reference memory once previously, but were found to have at best a modest impairment across five days of testing; moreover, while not stipulated by the authors, the MTT group had equivalent path-lengths to the control group by the final day of testing (Winter et al., 2011). Inconsistent results have been found following MB lesions, which have been shown to both disrupt (Sutherland and Rodriguez, 1989) and spare reference memory acquisition in the water maze (Santin, 1999). Unlike MTT lesions, ATN lesions typically result in severe place learning deficits in the water maze, which are often reminiscent to the effects of explicit hippocampal lesions (Warburton et al., 1999, Warburton & Aggleton, 1999; Wilton et al., 2001; Wolff et al., 2008a; 2008b, but see Sutherland & Rodriguez, 1989). Nonetheless, the evidence thus far has come from separate lesion studies and hence the effects of MTT and ATN lesions clearly require an explicit within-study comparison.

The standard reference memory task is not the only point of difference between MTT and ATN lesions. In separate studies from the same lab, ATN but not MTT lesions impaired relative length discriminations needed to locate a fixed platform in a modified water maze (Aggleton et al., 2009; Vann, 2013; Dumont et al., 2014). On this task MTT lesions were able to discriminate the fixed location of a hidden platform at an equivalent level to controls (Vann, 2013). By contrast, rats with ATN lesions discriminated between the four corners of the maze at chance levels (Dumont et al., 2014; Aggleton et al., 2009). This finding has led to

the suggestion that MTT lesions do not impair navigation per se, but rather may impair the ability to rapidly encode spatial information (Dillingham et al., 2015).

In terms of other tasks, neither MTT nor ATN lesions impair spontaneous object recognition (Aggleton et al., 1995; Warburton & Aggleton, 1999; Wilton et al., 2001; Moran & Dalrymple Alford, 2003; Dumont & Aggleton 2013; Nelson & Vann, 2014; 2016), but both lesions impair spontaneous object-in-place discriminations (Wilton et al., 2001; Nelson & Vann, 2014). In a different task MTT lesions, but not ATN lesions, impaired acquisition of a contextual discrimination involving visual contexts, but not an equivalent discrimination involving thermal contexts (Vann, Honey & Aggleton, 2003; Dumont, Amin & Aggleton 2014). Furthermore, rats with either MTT or ATN lesions show impaired discrimination of object location but not a task requiring non-spatial textural cues (Nelson & Vann, 2014; Dumont, Amin & Aggleton, 2014). In these studies, rats were impaired when required to discriminate one corner of a room from another for food reward irrespective of which direction they approached the object from at each location. In separate studies, rats with MTT and ATN lesions acquired this task more slowly and less accurately than sham controls, but were performing above chance by the end of testing (Dumont, Amin & Aggleton, 2014; Nelson & Vann 2014).

The ability to make arbitrary associations between items and spatial or contextual cues is another procedure that is regarded as akin to critical aspects of episodic memory (Preston & Eichenbaum, 2010). ATN lesions in rats have also been shown to disrupt the ability to make arbitrary (bi-conditional) associations between a spatial location and a specific odour in a go no-go procedure (Gibb et al. 2006). In object-place paired associate learning task, both ATN and hippocampal lesions result in severely impaired acquisition, with little improvement found even after 500 trials (Sziklas & Petrides, 1999; Sziklas et al., 1996). In this task rats had to discriminate between two distinct objects (presented simultaneously, as opposed to one item at a time used in the odour-place task of Gibb et al, (2006). That is, one of two objects was correct in spatial location A and the alternate object was correct in spatial location B. Surprisingly, rats with extensive MB lesions, which encompassed the most if not the entire mammillary region, acquired these object-place pairings at equivalent rate to shams (Sziklas et al., 1996). While MTT lesions might result in a more complete and selective removal of the MB influence to the extended hippocampal circuit (Dillingham et al., 2015), the lesions used by Sziklas et al, 1996) were large enough to cause severe impairments in the RAM, the difference between MB and ATN lesion effects



would be a highly important difference if replicated. Hence Chapter 7 addresses with important issue by examining the effects of MTT lesions, with proven deficits on the RAM, on an object-place task using an open test chamber.

The effects of MTT and ATN lesions on recency judgements or temporal context have also been examined (Nelson & Vann, 2016; Dumont & Aggleton, 2012). Deficits in memory for temporal contexts are a consistent neuropsychological finding in patients with Korsakoff's syndrome, and so might be particularly sensitive to damage in the MB-MTT-ATN axis (Kopelman, 2014; Nelson & Vann, 2016). Rats were required to discriminate between pairs of objects that had been presented in separate blocks of testing or within the same continuous block, but at different time points. MTT rats performed at chance level on both between, and within-block tests of recency memory (Nelson & Vann 2016). The authors suggested that this pattern of results indicate a specific problem in dealing with multiple items or events, possibly due to a heightened sensitivity to the effects of proactive interference (Nelson & Vann, 2016). However, a previous study showed that increasing proactive interference by using massed spatial alternation trials in the T-maze did not produce impairments in rats with MTT or MB lesions (Vann & Aggleton, 2003). Like MTT lesions, ATN lesions also impaired recency discrimination within a block, but unexpectedly enhanced recency discrimination between blocks (Dumont & Aggleton, 2012). It is unclear whether the different deficit profiles for MTT and ATN lesions on this task are robust or simply relate to subtle methodological differences. A previous study has also shown that ATN lesions impair temporal memory judgements of a within-block series of odours in a similar manner to the impairments shown by rats with hippocampal lesions (Wolff et al., 2006).

In addition to the tasks described above, ATN lesions have been tested on tasks not yet examined following MTT lesions and these are discussed below. In contrast to the consistent spatial working and reference memory deficits, rats with ATN lesions are not impaired in conditional and discriminative place learning tasks that are sensitive to hippocampal lesions. For example, ATN lesions do not impair conditional associative tasks that rely on egocentric discrimination, such as a cue signalling the rewarded arm of a T-maze, spatial discrimination in an operant box or configural learning in a water maze (Sziklas & Petrides 1999; 2004; 2007; Aggleton et al., 2009; Wolff et al., 2015). In a modified version of the water maze task, Moreau and colleagues (2013) presented visual cues within a water maze. ATN lesion rats were substantially impaired on the spatial discrimination task in which the specific visual cue was redundant but were not impaired on a visual pattern discrimination

task in which spatial location of the cue was redundant. This body of literature shows that memory deficits after ATN lesions are selectively found on tasks that require the use of externally-defined spatial representations.

Surprisingly, recent evidence suggests that instead of null effects ATN lesions may actually facilitate performance on certain tasks. Wright et al. (2015) examined whether the ATN were involved in attentional processes typically supported by the prefrontal cortex. In this study, rats with ATN lesions were tested on an attentional set shifting paradigm that measured the ability of the rats to pay attention to stimuli dimensions that reliably predict reinforcement (“intradimensional shift”), but also their ability to shift attention to another stimulus dimension when contingencies change (“extradimensional shift”). Wright et al. (2015), found that in stark contrast to the effects of prefrontal damage, ATN lesions impaired intradimensional shifts but facilitated extradimensional shifts. The authors suggested that this pattern of performance indicates that the ATN are critical for attending to those stimuli that are the best predictors of reward, but it is also possible that intact rats use spatial or other strategies that impede their performance and that ATN rats are less encumbered by such strategies.

Surprisingly, there is only one case of an MTT lesion being made in a non-human primate (Aggleton and Mishkin 1983). Aggleton & Mishkin (1983) were interested in the contributions of the anterior and posterior thalamus to memory and included a single monkey with an MTT lesions as a comparison. The monkeys were tested on a delayed non-matching to sample task, in which they had to distinguish between a novel and familiar objects to obtain a reward. The monkey with the MTT lesion displayed a clear, but moderate impairment that became more apparent when the time between object presentations or the length of the object lists was increased (Aggleton & Mishkin, 1983). Perhaps more importantly, this deficit was comparable to other monkeys with lesions to the anterior portion of the thalamus, which suggests an important role for the MTT and MB in modulating thalamic function. However, no data for the monkey with the MTT lesion was reported for the additional tasks performed by the thalamic lesion groups, restricting more complete conclusions. In a more recent study using non-human primates, selective lesions to the anterior thalamic nuclei (rather than the anterior portion of the thalamus) substantially impaired acquisition of a visual object-place memory task, a task designed to capture the ‘whole scene’ nature of episodic memory (Parker & Gaffan, 1997)

One notable consequence of MTT lesions in rats is retrograde degeneration of the mammillary bodies (Vann & Aggleton 2003; Vann 2013). This MB atrophy is suggested to be the result of neuronal loss, but is yet to be formally examined (Vann, 2013). In the CNS, an axotomy or the removal of the primary projection target of an axon, typically induces a progressive functional decline in the originating cell, causing cellular atrophy and eventually cell death (Barron, 2004). Previous research has also shown that lesions to the anterior, but not posterior medial thalamus in monkeys resulted in substantial degeneration and gliosis in both the MB and medial dorsal nucleus of the thalamus. In all three cases the MTT was also interrupted, but the extent of the lesions mean that the reasons behind neuronal loss in the MB are unclear (Aggleton & Mishkin 1983). Therefore examining cell loss in the MB with a neuron specific marker may provide a measure of the relative influence of ATN and MTT lesions on MB integrity. These findings could provide additional information regarding the importance of the brainstem input for ATN function i.e. greater MB degeneration after ATN than MTT lesions would suggest a more profound deficit and point to the influence of the brainstem-MB pathway for normal memory function.

### 4.3. Summary of MTT and ATN lesion studies

Looking across separate MTT and ATN lesion studies both lesions reliably result in spatial working memory deficits, although ATN lesion deficits are often more severe especially in the T-maze. It is uncertain, however, whether both lesions impair the ability to learn associations between a stimuli and a spatial or temporal context, which is consistent with findings from patients with Korsakoff syndrome. Another point of difference seems also to be reference memory in the context of place learning. ATN lesions reliably disrupt the acquisition of spatial reference memory, but a mild deficit at best was found after MTT lesions in the only study that examined this task. In general, the limited MTT literature and the lack of a direct comparison between the MTT and ATN, which could control for subtle differences between studies, makes definitive conclusions premature at this stage. The next section discusses the behavioural impact of lesions to the mammillary body lesions and their primary inputs, the VTg and pFx, which is necessary in order to understand the influence of the MTT on the extended hippocampal circuit.

**Table 4.1: MTT lesion behavioural studies**

Year	Authors	Lesion site/s	Lesion method	Behavioural tasks	Deficits
2016	Nelson & Vann	MTT Contra MTT & PFC	Radio Frequency	1. Multi-item recency judgements 2. Single-item recency 3. Object recognition	1. MTT impaired, MTT-PFC not impaired 2. MTT,MTT-PFC not impaired 3. MTT,MTT-PFC not impaired
2014	Nelson & Vann	MTT	Radio frequency	Object tasks 1. Location discrimination 2. Object recognition 3. Object in place Radial arm maze 4. Standard task 5. mid trial rotation	1. MTT impaired, but above chance performance 2. MTT not impaired 3. MTT impaired, did not discriminate 4. MTT impaired 5. MTT greater impairment than std task
2013	Vann	MTT VTg pFx	Radio frequency, NMDA	T-maze 1. DNMP 2. DNMP two mazes 3. DNMP two mazes in the dark Radial arm maze 4. working memory 5. mid trial rotation Water maze 6. geometric discrimination	1.VTg mildly impaired; MTT & pFx not impaired 2. MTT & VTg equivalently impaired, pFx not impaired 3. MTT & VTg equivalently impaired, pFx not impaired 4. MTT & VTg equivalently impaired, pFx not impaired 5. MTT & VTg impaired, pFx not impaired 6. MTT, VTg and pFx not impaired
2011	Winter, Wagner et al	MTT	Electrolytic	1. Food Hoarding Paradigm Water maze 2. reference memory 3. working memory	1. MTT impaired, but only when using self-movement cues 2. MTT mild transient impairment 3. MTT not impaired

Year	Authors	Lesion site/s	Lesion method	Behavioural tasks	Deficits
2003	Vann & Aggleton	MTT, MB's	Radio frequency, NMDA	T-maze 1. DNMP Radial Arm Maze 2. standard working memory task Water maze 3. DNMP	1. MTT & MB impaired, but only transiently 2. MTT impaired, but not MB 3. MTT & MB impaired, MTT tended to perform worse than MB
2003	Vann, Aggleton & Honey	MTT	Radio frequency	Contextual discrimination 1. Spatial cues 2. Thermal cues	1. MTT impaired acquisition 2. MTT not impaired
1985	Thomas & Gash	MTT	Electrolytic	T-maze 1. DNMP	1. MTT transient deficit
1978	Field, Rosenstock, King & Greene	MTT MTG MB	Electrolytic	1. T-maze, massed trials 2. Ambulation in the open field	1. MTT, MTg and MB impaired. MTT made the most errors followed by MTg and then MB. 2. MTT equivalent to control, but MTg and MB hyperactive
1968	Kriekhaus & Randall	MTT	Electrolytic	1. T-maze	1. MTT not impaired

**Abbreviations:** DNMP = delayed non-matching to place; MTT = mammillothalamic tract; MB = mammillary bodies; MTG = mammillotegmental tract; NMDA = N-methyl-d-aspartate; pFx = post-commissural fornix.

**Table 4.2: ATN lesion behavioural studies from the past two decades**

Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
2016	Alcaraz, Naneix, Defosses, Marchand, Wolff & Coutureau	ATN MD	NMDA	T-maze 1. Goal oriented spatial behaviour 2. DNMP Operant box 3. progressive ratio	1. ATN and MD impaired 2. ATN impaired, MD not impaired 3. ATN and MD not impaired
2015	Wolff, Faugere, Defosses, Coutureau & Marchand	ATN MD	NMDA	1. Conditional discrimination task	1. ATN not impaired during acquisition and reversal. MD impaired during acquisition, but not reversal
2015	Wright, Vann, Aggleton and Nelson	ATN	NMDA	1. Set shifting	1. ATN impaired during acquisition of intra-dimensional shifts, but enhanced performance during extradimensional shifts
2014	Dumont, Amin & Aggleton	ATN	NMDA	1. Biconditional discrimination 2. Biconditional learning (context/place) 3. Spatial bi/unidirectional discrimination 4. place biconditional discrimination	1. ATN not impaired 2. ATN impaired on place only 3. ATN impaired 4. ATN impaired
2014	Dumont, Wright, Pearce & Aggleton	ATN	NMDA	1. Passive geometric place learning 2. Passive/active place learning (colour arrangement) 3. T-maze alternation 4. Passive/active place learning (cue arrangement)	1. ATN impaired 2. ATN initial acquisition impairment 3. ATN impaired 4. ATN initial acquisition impairment

Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
2014	Ulrich, Aitken, Abraham, Dalrymple-Alford & McNaughton	ATN	NMDA	T-maze spatial working memory 1. 1-week training break relearning 2. 15-week training break relearning	1. ATN impaired 2. ATN impaired
2014	Harland, Collings, McNaughton, Abraham & Dalrymple-Alford	ATN	NMDA	1. Cross-maze spatial working memory 2. RAM spatial reference working memory	1. ATN impaired 2. ATN impaired
2013	Dumont & Aggleton	ATN	NMDA	1. T-maze spatial alternation 2. Bow-tie maze object recognition 3. Object recency between-block 4. Object recency within-block 5. Odour recognition 6. Odour recency between-block 7. Object recognition open arena 8. Locomotor activity	1. ATN impaired 2. ATN not impaired 3. ATN not impaired 4. ATN impaired 5. ATN not impaired 6. ATN showed mild impairment in discrimination between sample phases 7. ATN not impaired 8. ATN showed greater hyperactivity
2013	Mendez-Lopez, Arias, Bontempi & Wolff	ATN	NMDA	1. Radial arm maze (8-arm) spatial discrimination	1. ATN impaired
2013	Moreau et al	ATN, ILN-LT	NMDA	Water maze 1. spatial discrimination 2. Visual pattern discrimination	1. ATN impaired, ILN-LT not impaired 2. ATN and ILN-LT not impaired
2011	Aggleton, Amin, Jenkins, Pearce & Robinson	ATN	NMDA	T-maze 1. Spatial alternation 2. Sequence learning	1. ATN impaired 2. ATN not impaired

Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
2010	Dumont, Petrides & Sziklas	Fornix-RSC ATN-H-RSC-Ipsi ATN-H-RSC-Contra	RF and/or ibotenic	1. Spatial-visual conditional associative learning 2. Radial arm maze (8-arm) spatial working memory	1. Fornix-RSC, ATN-H-RSC-Contra severely impaired, ATN-H-RSC-Ipsi Impaired 2. Fornix-RSC, ATN-H-RSC-Contra and Ipsi impaired
2009	Aggleton et al., (2009)	Fx ATN	RF NMDA	1. Configural learning 2. Geometric learning 3. T-maze spatial alternation	1. ATN and Fx not impaired 2. ATN impaired, Fx not impaired 3. ATN and Fx impaired
2009	Lopez et al	ATN, ILN-LT	NMDA	Water maze 1. Spatial reference memory 2. recent (5 days) probe 3. Remote (25 days) probe	1. ATN impaired, ILN-LT not impaired 2. ATN impaired due to failure on #1, ILN-LT not impaired. 3. ILN-LT impaired, ATN impaired due to failure on #1
2008	Wolff, Loukavenko, Will & Dalrymple-Alford	ATN	NMDA	Water maze spatial reference memory 1. fixed release point 2. variable start points	1. ATN impaired acquisition 2. ATN impaired acquisition
2008	Wolff, Gibb, Cassel & Dalrymple-Alford	ATN, ILN	NMDA	1. water maze spatial reference memory 2. radial arm water maze (8 arm) egocentric spatial memory	1. ATN impaired, ILN not impaired 2. ATN and ILN not impaired
2007	Loukovenko, Ottley, Moran, Wolff & Dalrymple-Alford	ATN	NMDA	Cross-maze spatial working memory 1. 14 days post-surgery 2. 75 days post-surgery 3. 120 days post-surgery	1. ATN severely impaired 2. ATN severely impaired 3. ATN severely impaired



Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
2007	Sziklas & Petrides	ATN	Electrolytic	1. Visual-spatial conditional associative task 2. Radial arm maze (8 arm) spatial working memory	1. ATN not impaired 2. ATN impaired
2006	Frohardt, Bassett & Taube	AD, DTN	NMDA	1. Path integration food carrying, visual/blindfolded	1. AD mildly impaired, DTN severely impaired
2006	Gibb, Wolff and Dalrymple-Alford	ATN, MT, LT	NMDA	1. Odour-place paired associative task 2. Odour discrimination 3. Spatial discrimination	1. ATN and LT impaired, MT not impaired 2. ATN impaired acquisition, LT and MT not impaired 3. ATN impaired acquisition, LT and MT not impaired
2006	Mitchell & Dalrymple-Alford	ATN, LT	NMDA	1. Elevated plus maze: response working memory 2. Radial arm maze (8 arm) spatial working memory	1. ATN impaired and LT impaired 2. ATN and LT not impaired
2006	Wolff, Gibb and Dalrymple-Alford	ATN	NMDA	1. temporal order memory for odour sequences 2. Odour recognition memory 3. Task-reversal	1. ATN impaired 2. ATN not impaired 3. ATN not impaired
2004	Henry, Petrides, St-Laurent & Sziklas	ATN-HPC-Contra	Ibotenic	1. Visuospatial conditional associative learning 2. Forced spatial alternation with delay	1. ATN-HPC-Contra impaired 2. ATN-HPC-Contra impaired
2004	Sziklas & Petrides	ATN, HPC, MB	Electrolytic	1. Egocentric visual-spatial conditional associative learning	1. HPC impaired, but ATN and MB not impaired
2003	Mair, Burk & Porter	ATN, PH, ATN-PH	NMDA and radiofrequency	1. Radial arm maze (8 arm) DNMP	1. ATN, PH comparable deficits, ATN-PH delay dependent deficits ATN delay dependent deficits at 5-6 weeks post-surgery

Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
2003	Moran & Dalrymple-Alford	ATN, PRC	NMDA	1. Spontaneous object recognition Radial arm maze (12-arm) 2. Spatial working memory 3. delay task 4. Elemental cue task 5. Configural cue task	1. ATN and PRC not impaired 2. ATN impaired, PRC not impaired 3. ATN impaired, PRC not impaired 4. PRC impaired, ATN not impaired 5. PRC impaired, ATN not impaired
2002	Van Groen, Kadish & Wyss	AD/AV, AD/AV+ AD,AV,AM	Ibotenic	Water maze 1. Spatial working memory 2. Immediate probe	1. AD/AV mild impairment, AD/AV+ impaired, AD, AV, AM severe impairment 2. AD/AV mild impairment, AD/AV+ impaired, AD, AV, AM severe impairment
2001	Alexinsky	ATN, MD RSC, PPC	Ibotenic Excision	1. RAM (8 arm) spatial reference working memory 2. RAM(8 arm) spatial reference working memory, new spatial location 3. Contextual change	1. ATN and MD impaired, RSC and PPC not impaired 2. ATN, MD and PPC impaired but not RSC 3. Only ATN impaired
2001	Warburton, Baird, Morgan, Muir & Aggleton	ATN-HPC- Contra ATN-HPC- Ipsi HPC+	NMDA	1. T-maze spatial forced alternation 2. Water maze spatial reference memory 3. RAM (8 arm) spatial working memory	1. ATN-HPC-Contra impaired, ATN-HPC-Ipsi not impaired 2. ATN-HPC-Contra impaired, ATN-HPC-Ipsi not impaired 3. ATN-HPC-Contra impaired, ATN-HPC-Ipsi not impaired

Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
2001	Wilton, Baird, Muir, Honey & Aggleton	AD- LD	NMDA	1. T-maze spatial forced alternation 2. water maze spatial working memory 3. spontaneous object recognition 4. Object-in-place	1. AD-LD impaired 2. AD-LD impaired 3. AD-LD not impaired 4. AD-LD impaired
1999	Sziklas & Petrides	ATN	Electrolytic	1. Object-place paired associate task 2. RAM (8-arm) spatial working memory 3. T-maze conditional egocentric task	1. ATN impaired 2. ATN impaired 3. ATN not impaired
1999	Warburton & Aggleton	ATN Fx	NMDA Radiofrequency	1. Water maze spatial reference memory 2. T-maze spatial forced alternation 3. Object recognition	1. ATN and Fx impaired 2. ATN and Fx impaired 3. ATN and Fornix not impaired
1999	Warburton, Morgan, Baird, Muir & Aggleton	ATN Fx ATN+	NMDA Radiofrequency NMDA	1. water maze spatial reference memory 2. T-maze forced alternation	1. ATN and fornix comparable impairments, ATN+ severely impaired 2. ATN and fornix comparable impairments, ATN+ severely impaired
1997	Warburton, Baird & Aggleton	ATN ATN+LD Fx	NMDA NMDA Radiofrequency	1. T-maze spatial forced alternation 2. Cross maze allocentric alternation 3. Egocentric discrimination	1. ATN, Fx and ATN-LD impaired acquisition 2. ATN, Fx and ATN-LD impaired 3. ATN, Fx and ATN-LD not impaired
1996	Byatt & Dalrymple-Alford	AV AM	Radiofrequency	1. RAM (12-arm) spatial reference and working memory	1. Both AV and AM impaired

Year	Authors	Lesion site	Lesion method	Behavioural task	Deficits
1996	Aggleton, Hunt, Nagle and Neave	AV/AD, AM, ATN.T	NMDA	1. T-maze spatial forced alternation 2. cross-maze allocentric alternation 3. RAM (8 arm) spatial working memory 4. Egocentric discrimination	1. AM and AV/AD impaired acquisition ATN.T impaired 2. AM and AV/AD not impaired ATN.T impaired 3. AV/AD mild impairment, AM not impaired, ATN.T impaired 4. ATN.T, AV/AD and AM not impaired
1995	Aggleton, Neave, Nagle & Hunt	ATN MB Fx	NMDA NMDA Radiofrequency	1. T-maze spatial forced alternation 2. object recognition	1. Fx and ATN lesions severely impaired acquisition, MB lesions impaired acquisition. Delay dependent deficits more pronounced in Fx group. 2. ATN, MB and Fx not impaired

**Abbreviations:** AD = anterodorsal nuclei; AM = anteromedial nuclei; ATN = anterior thalamic nuclei; ATN.T = total ATN lesions; AV = anteroventral nuclei; Contra = contralateral; DTN = dorsal tegmental nucleus; Fx = fornix; ILN = intralaminar nuclei; Ipsi = ipsilateral; LD = laterodorsal nucleus; LT = lateral thalamus; MB = mammillary bodies; MT = medial thalamus; PH = parahippocampal cortex; PRC = perirhinal cortex; RAM = radial arm maze; RF = radiofrequency lesion; RSC = retrosplenial cortex.

#### 4.4. MB lesions

The effects of MB damage (summarized in table 4.3) are typically less severe than ATN damage, which may in part be related to the difficulty of making sufficiently large, yet selective MB lesions (Aggleton, 2008). The resulting deficit of MB lesions also seems to diminish with training although possibly through the use of alternative routes/strategies, such as through the direct connections from the hippocampus to the ATN via the fornix. Like total ATN lesions, deficits after large MB lesions are suggested to be a result of the loss of both head direction information and the disruption of theta rhythms through separate pathways mediated by the lateral and medial MB respectively (Vann & Aggleton 2004). However, lesion studies specifically targeting the lateral MB head direction circuit have found mild or no deficits on both spatial working and reference memory tasks (Harland et al., 2015; Vann, 2005). These findings suggest that damage to the medial MB might be primarily responsible for MB deficits. In line with this premise, a study injecting retrograde tracer into the ATN found that even postero-ventral MTT lesions selectively removed the influence of the medial, but not lateral MB (Vann & Albasser, 2009). This work suggests that the loss of rhythmic oscillatory activity from the medial MB, rather than head direction information from the lateral MB to the ATN, might be critically important for spatial memory processing, although the additional contribution from loss of head direction signals may be important in some instances.

A limited amount of studies have examined memory function after either full or partial MB lesions in monkeys, and these have not all produced consistent deficits (Holmes et al., 1983; Aggleton & Mishkin, 1985; Zola-Morgan, 1989; Parker & Gaffan, 1997). Looking across these studies, MB lesions produced the greatest impairments on tasks taxing spatial memory processing (Holmes et al, 1983; Aggleton & Mishkin, 1985). In terms of other tasks, one study reported that monkeys with medial MB lesions were unimpaired on a delayed non-matching to sample object recognition task (Aggleton & Mishkin, 1985). However, more complete MB lesions produced a transient impairment on this same task in a different study (Zola-Morgan, 1989). Additional tasks requiring simple visual discriminations or recalling which of two locations contained a food reward do not appear to be impaired by MB lesions. The more recent study with monkeys found that bilateral MB lesions severely impaired learning an automated object-in-place memory task (Parker & Gaffan, 1997). This task required the monkeys to form an association between an object and a specific location within a digital scene. Monkeys with MB lesions made considerably more errors across trials, and

were found to have equivalent performance to monkeys with fornix transection (Parker & Gaffan, 1997). Furthermore, subsequently giving an additional fornix lesion to the monkeys did not increase the severity of this deficit. These authors suggested that, at least for this task, the behavioural deficit likely reflects the loss of the fornical input into the MB, to explain the lack of effect of the additional fornix lesion (Parker & Gaffan, 1997).

Historically the perceived contributions from the MB have been dominated by a focus on their hippocampal inputs via the fornix (Dillingham et al., 2015). If this line of thinking is correct then MTT lesion deficits would relate to the loss of both the direct inputs and indirect inputs, via the MB, from the hippocampal formation to the ATN. In support of this position, Wright et al., (2010) reported that the connections from the subiculum to the MB and ATN arise from two segregated populations of subicular neurons. The authors suggest that the topographical organisation of the subicular neurons projecting to the MB and ATN are potentially capable of providing two independent streams of information, both of which might be important for spatial memory processing (Wright et al., 2010).

To explicitly examine the importance of the fornix inputs in to the MB, Vann et al. (2010) transected the descending portion of the post-commissural fornix (pFx), which selectively disconnects the subiculum input to MB but leaves intact the direct connection between the subiculum to the ATN. It is not possible to selectively disconnect the latter input using tract-based lesions. If the MB functions solely as a hippocampal relay then severing the pFx would induce a comparable behavioural deficit to MB and MTT lesions. Discounting this hypothesis, however, Vann et al., (2010) found rats with pFx lesions only had a mild spatial alternation deficit in the T-maze, and no apparent deficits in the standard working memory versions of the water-maze and radial arm maze. Hence, the authors suggested that the MB and MTT appear to have critical, independent non-hippocampal contributions to the extended hippocampal memory system.

#### 4.5. Ventral tegmental Nucleus of Gudden (VTg)

The minimal spatial memory impairment observed following pFx lesions placed emphasis on the other prominent input in to the MB, the tegmental nucleus of Gudden, particularly the VTg (Vann, 2009). As previously discussed, this nucleus has dense reciprocal connections with the medial MB. Unlike pFx lesions, VTg lesions in rats produce deficits on the same array of spatial working memory tasks and to a similar degree as MB/MTT lesions. These

findings suggest that VTg and not descending fornix afferents to the MB are critical for normal memory function. Unfortunately, there is only one reported case of a man with amnesia that was attributed to pathology in the VTg region (Goldberg et al, 1981).

More recently, Vann (2013) explicitly compared the effects of lesions to the two major MB inputs, the pFx and VTg, with the major MB output, the MTT within the same study. These groups were contrasted across spatial working memory tasks in the T-maze, radial arm maze and water maze. This design allowed Vann (2013) to determine which of primary MB inputs best approximated the MTT lesion deficit. Because of subtle procedural differences between studies explicit within-study comparison is necessary to establish the relative contribution of any individual structure. The results of this experiment were clear. Lesions to the VTg produced an equivalent deficit to MTT lesions across tasks in the T-maze and RAM (Vann, 2013). By contrast, rats with lesions to the pFx were not impaired. These results indicate that the MB/MTT influence on the ATN is provided primarily by brainstem and not hippocampal inputs.

The next section examines the evidence of functional changes in distal sites within the extended hippocampal circuit following lesions to the diencephalon. Most of these studies have found hypoactivation of immediate early genes especially in the retrosplenial cortex. These “functional lesions” have been proposed to contribute to the lesion related deficits.

**Table 4.3: MB lesion behavioural studies**

Year	Authors	Lesion site/s	Lesion method	Behavioural tasks	Deficits
2015	Harland, Wood & Dudchenko	LMN	Ibotenic acid	1. Spatial landmark task Water maze 2. Reference memory 3. Reversal task	1. LMN not impaired 2. LMN not impaired 3. LMN impaired
2011	Vann	LMN	Ibotenic acid	T-maze alternation 1. Standard task 2. Two mazes 3. Two mazes in the dark Water maze 4. Geometric learning	1. LMN not impaired 2. LMN impaired 3. LMN not impaired 4. LMN initially impaired
2005	Vann	LMN	Ibotenic acid	1. T-Maze WM 2. Water maze WM	1. LMN not impaired 2. LMN impaired
2001	Gaffan, Bannerman, Warburton, & Aggleton	Fx MB ATN RH	Severed NMDA NMDA NMDA	1. T-maze alternation 1. Visual scene discrimination	1. ATN & MB impaired. ATN performed at chance level (51%) whereas MB (83%). Other lesions were not tested on this task. 2. RH not impaired. Fx, ATN and MB enhanced performance
1999	Santin, Rubio, Begega, & Arias	MMn	Electrolytic	Water-maze 1. Reference memory 2. Transfer test 3. reversal learning 4. Visual	1. MB not impaired
1997	Neave, Nagle, & Aggleton	MB Fx CCB	NMDA, Radio frequency	1. T-maze, 2. Cross maze 3. RAM	1. MB, FX and CCB impaired 2. MB impaired, FX and CCB not impaired 3. MB, FX and CCB impaired



Year	Authors	Lesion site/s	Lesion method	Behavioural tasks	Deficits
1994	Harper, Mclean & Dalrymple-Alford	MB MS	Radio frequency	1. DNMS in an operant chamber	1. MS, but not MB lesions impaired performance
1993	Harper, Dalrymple-Alford & Mclean	MB MS	Radio frequency	RAM (12 arms) 1. Serial arm recognition	1. MS impaired, MB lost primacy and recency effect but no impairment overall.
1993	Sziklas & Petrides	MB-R HPC	Electrolytic	1. T-maze conditional association 2. RAM 3. Object-place paired associate task	1. HPC impaired, MB-R not impaired 2. MB-R and HPC impaired 3. HPC impaired, MB-R not impaired
1993	Sziklas & Petrides	MB MB-R	Electrolytic	1. RAM 2. RAM extended ITI 3. non-spatial DNMS 4. Conditioned taste aversion	1. MB & MB-R no impairment 2. MB no impairment, MB-R impaired 3. MB & MB-R no impairment 4. MB & MB-R no impairment
1990	Beracochea, & Jaffard	MB	Ibotenic acid	T-Maze 1. Spontaneous Acquisition 2. Sequential delayed alternation	1. MB impaired 2. MB not until ITI's extended from 50s to 3mins
1989	Sutherland & Rodriguez	FFC ATN NAcc MS MB	Electrolytic	Water-maze, Reference memory 1. Retention 2. Acquisition	1. Only FFC impaired 2. FFC, NAcc and ATN impaired, MB and MS both moderately impaired

Year	Authors	Lesion site/s	Lesion method	Behavioural tasks	Deficits
1987	Beracochea & Jaffard	MM	Radio Frequency, or Kainic acid	T-Maze 1.Spontaneous Alternation 2.S.A reduced ITI 30s to 5sec 3.Adding external cue	1. MM impaired 2. MM not impaired 3. MM not impaired
1984	Jarrad, Okaichi, Steward & Goldschmidt	FF EC DG MB	Electrolytic Electrolytic Colchicine Electrolytic	RAM 1. Place task 2. Cue task	1. FF and EC impaired, DG and MB not impaired 2. FF and EC impaired, DG and MB not impaired

**Abbreviations:** A = amygdala; ATN = anterior thalamic nuclei; CCB = cingulum bundle; DG = dentate gyrus; DNMS = delayed non-matching to sample; EC = entorhinal cortex; FF = fimbria fornix; Fx = fornix; HPC = hippocampus; LMn = lateral mammillary nuclei; MB = mammillary bodies; MB-R= mammillary body region (includes supramammillary nucleus and fibre tracts); MMn= medial mammillary nucleus; MS = medial septum; NAcc = nucleus accumbens; NMDA= N-Methyl-D-Aspartic acid (neurotoxin); RH = retrohippocampal region; RAM = radial arm maze.

#### 4.6. Downstream functional changes following diencephalic lesions

Another interesting feature of diencephalic lesions is that they consistently produce covert pathology in other distal regions of the extended hippocampal system. Covert pathology refers to an area that appears normal by standard histological means but shows evidence of a functional lesion (Aggleton, 2008). The RSC cortex seems especially sensitive to lesions in the diencephalon which consistently produce a striking reduction in one biomarker of covert pathology, immediate early gene (IEG) expression in the RSC. The RSC has major direct and indirect connections with the ATN and hippocampus (Aggleton, 2008). Less consistent changes in IEG's are found in the hippocampus, suggesting that these changes might be more marker and task specific.

IEG's are a class of transcription factors that are rapidly encoded after a cell is activated (Davis et al., 2003). Although this observation has not yet been directly linked to any changes in behaviour, increases in the RNA levels of three IEGs (zif268, c-fos and Arc) have been observed in the dorsal hippocampus following training in a spatial water maze task (Guzowski et al. 2001), and the presence of CREB and c-Fos have been associated with improved spatial memory performance (Czajkowski et al., 2014). Additionally, some IEGs appear to have important roles in consolidating long-term memory and hippocampal plasticity. For example, zif268 overexpression in mice has been shown to increase long-term potentiation in the dentate gyrus, and this was paralleled by an enhanced ability to form a long-term memory of the spatial location of an object (Penke et al., 2014). This finding suggests that zif268 might be especially relevant to learning and memory processes.

Table 4.4 provides a summary of studies that have assessed the levels of IEG activation in rats after MTT and ATN lesions, most of which focus on zif268 and c-fos. Of note is that both MTT and ATN lesions consistently produce marked reductions of c-fos (figure 4.2) and zif268 across the subregions of the RSC (Jenkins et al., 2002; Vann & Albasser, 2009; Dumont et al., 2012; Dupire et al., 2013; Frizzarti et al 2016; Loukavenko et al., 2016). This effect is of particular note after MTT lesions, as the MTT is only indirectly connected to the RSC via the ATN, which provides strong evidence for widespread functional changes to memory structures following diencephalic damage that does not rely simply on a disconnection effect (Vann & Albasser, 2009; Vann, 2013). If considered separately, both MTT and ATN lesions tend to have a more extensive impact on c-fos immunoreactivity, than zif268. For example, in addition to retrosplenial cortex changes both

MTT and ATN lesions reduced c-fos expression in the hippocampus, prelimbic cortex and anterior cingulate cortex in some studies (Jenkins et al., 2002a,b; Vann & Albasser, 2009; Dupire et al., 2013; Vann 2013; but see Loukavenko et al, 2016 and Dupire et al, 2013).

**Table 4.4: Summary of studies that examined biomarkers of neural activation following MTT (first) or ATN (subsequent) lesions**

Year	Authors	Lesion type	Behavioural Deficits	Neural activity marker induction procedure	Neural activity marker measure and regions analysed	Outcome relative to neural activity marker
2016	Frizzati, Milczarek, Sengpiel, Thomas, Dillingham and Vann	MTT	MTT lesions severely impaired T-maze alternation (only cohort 2 tested)	Forced runs in a radial arm maze (8-arm) in a novel room with novel cues.(cohorts 1 and 2)	Zif268 (cohort 1) and Cytochrome oxidase (cohort 2) examined in the DG, CA1, CA3 and RSC (sup and deep)	MTT lesions reduced zif268 in the sup. and deep laminae of the Rgb and Rdg. Reduced cytochrome oxidase in the sup. Rdg and the deep Rga and Rgb.
2013	Vann	MTT PFx VTg	MTT lesions impaired T-maze alternation when intra-maze cues were minimised.	Forced runs in a radial arm maze (8-arm) in a novel room with novel cues.	c-Fos was examined in the RSC, HPC, IL, PL, somatosensory cortex, SUM, LS and MS	MTT lesions reduced Fos counts in the RSC, HPC and PL.
2009	Vann and Albasser	MTT	Forced runs hence no behavioural difference.	Forced runs in a radial arm maze (8-arm) in a novel room with novel cues.	c-Fos was examined in the somatosensory, auditory and visual cortex, the RSC (sup. and deep), DG, CA3, CA1, dHPC, vHPC, lEnto, mEnto, PRC, Postrh, PL, IL, AC, dSub, vSub, cSub, Para, Pre and post subiculum.	MTT lesions reduced c-Fos counts in the sup. Rdg,Rgb and Rga as well as the deep Rdg and Rgb, PL, DG,CA3, CA1, dHPC and the para and post subiculum.

Year	Authors	Lesion type	Behavioural Deficits	Neural activity marker induction procedure	Neural activity marker measure and regions analysed	Outcome relative to neural activity marker
2015	Loukavenko, Wolff, Poirier & Dalrymple-Alford	Bilateral ATN lesions	ATN lesions severely impaired t-maze alternation	T-maze with 90 min in a dark room after each trial (for 3 trials)	c-Fos examined in the PL,IL, ACC, somatosensory and motor cortex, dHPC and RSC	ATN lesions reduced Fos counts in the sup. and deep Rgb.
2013	Dupire et al	Bilateral ATN lesions	ATN lesions reduced anxiety responses in the elevated plus maze, increased activity and reduced corticosterone levels when exploring an open field, and delayed acquisition of a conditioned contextual fear response.	Delay of 60 min between final contextual fear task and sacrifice.	c-Fos and pCREB examined in the amygdala (BLA and LA), HPC, subiculum and RSC (sup. and deep)	ATN lesions reduced Fos and pCREB counts in the BLA and sup. and deep laminae of the Rgb. Fos counts were also reduced in the ventral subiculum and sup. lamina of the ACC pCREB reduced in dCA1 And vCA1
2013	Mendez-Lopez, Arias, Bontempi and Wolff	Bilateral ATN lesions	ATN lesions severely impaired spatial discrimination in the radial arm maze.	Delay of 90 minutes between final testing session in the RAM and sacrifice.	Cytochrome oxidase examined in the PL,IL, ACC, RSC (sup. and deep). Pt, Ento, CPu, CA1, CA3, DG, Sub.	ATN lesions reduced Cox activity in the sup. lamina of the Rgb and the sup. lamina of the Cg1 region of the ACC.

Year	Authors	Lesion type	Behavioural Deficits	Neural activity marker induction procedure	Neural activity marker measure and regions analysed	Outcome relative to neural activity marker
2012	Dumont, Amin, Poirier, Albasser and Aggleton	<ol style="list-style-type: none"> <li>1. Unilateral ATN lesions (split into novel and familiar object groups)</li> <li>2. Bilateral ATN lesions</li> </ol>	<ol style="list-style-type: none"> <li>1. Novel group showed improved recency discrimination</li> <li>2. Forced choices in the RAM, thus no group difference</li> </ol>	<ol style="list-style-type: none"> <li>1. Novel and familiar object exploration</li> <li>2. Forced runs in a radial arm maze (8-arm) in a novel room with novel cues.</li> </ol>	<ol style="list-style-type: none"> <li>1. zif268 was examined in the PL, IL, PRC, HPC, dSub and post-sub, Rdg, Rgb and Rga.</li> <li>2. zif268, CREB, pCREB and GAP-43 in the same regions</li> </ol>	<ol style="list-style-type: none"> <li>1. Reduced zif268 counts in Rgb and post-sub. Novel object associated with changes in HPC zif268.</li> <li>2. Reduced zif268 counts in the Rgb and post-sub, and reduced pCREB counts in Rgb.</li> </ol>
2009	Poirier and Aggleton	<ol style="list-style-type: none"> <li>1. Unilateral ATN lesions</li> <li>2. Bilateral ATN lesions</li> <li>3. Unilateral ATN lesions</li> <li>4. Unilateral LD lesions</li> </ol>	Bilateral ATN lesions (2) were hyperactive when compared to sham rats	<ol style="list-style-type: none"> <li>1. Novel room and cage sacrificed at 1, 2, 4 and 8 weeks.</li> <li>2 (sacrificed at 4 weeks or 1 year), 3 (sacrificed at 4 weeks) and 4 (sacrificed at 3.5-4.5 months) Novel room with activity cages with beams for locomotor activity</li> </ol>	<ol style="list-style-type: none"> <li>1. c-Fos and zif268 in Rgb (sup. and deep)</li> <li>2 and 3. C-Fos in the Rgb and Rdg (sup. and deep)</li> <li>4. c-Fos and zif268 in the Rdg and Rgb (sup. and deep).</li> </ol>	<ol style="list-style-type: none"> <li>1. Greatest zif268 and c-Fos hypoactivity in the 1-week group, primarily in sup. laminae. Deep laminae counts were reduced only after week 8 in Fos and only week 4 in zif.</li> <li>2. Reduced c-Fos counts in sup. Rgb only after 4 weeks. Reduced c-Fos counts in the sup. Rgb and Rdg and deep Rdg after 1 year.</li> </ol>

Year	Authors	Lesion type	Behavioural Deficits	Neural activity marker induction procedure	Neural activity marker measure and regions analysed	Outcome relative to neural activity marker
						3. Reduced c-Fos counts in the sup. and deep Rdg and Rgb. 4. No change in c-Fos and Zif268 counts.
2008	Poirier et al	Unilateral ATN lesions		Novel room and cage with visual stimuli	c-Fos and multiple other transcription factors examined in the Rgb region	Reduced c-Fos and multiple transcription factors in the Rgb on lesion side relative to the intact side.
2004	Jenkins, Vann, Amin & Aggleton	1 & 2. Bilateral ATN lesions. 2. Postrhinal cortex lesions		1. Foraging in novel room 2. Activity box in a novel room	1. c-Fos examined in the subregions of the RSC 2. c-Fos and zif268 examined in the same regions	1 & 2. ATN lesions reduced c-Fos counts in the sup. laminae Rga and Rgb. 2. ATN lesions reduced c-Fos and zif268 counts in the sup. Rga, Rgb and the deep Rgb and Rdg. No changes following post rhinal lesions



Year	Authors	Lesion type	Behavioural Deficits	Neural activity marker induction procedure	Neural activity marker measure and regions analysed	Outcome relative to neural activity marker
2002	Jenkins, Dias, Amin & Aggleton	Unilateral ATN lesions	Unilateral ATN lesions did not impair spatial working memory in a radial arm maze (8 arm)	Spatial working memory task in the radial arm maze placed in dark box following trial	c-Fos was examined in the HPC, subicular, limbic and parahippocampal cortices on the lesion side compared to the intact side	Reduced c-Fos counts were found in the post and pre-sub, DG, dHPC, CA1, and RSC in the lesioned hemisphere.
2002	Jenkins, Dias, Amin, Brown & Aggleton	Bilateral ATN lesions	ATN lesions impaired spatial alternation in a T-maze and a spatial working-reference memory task in the radial arm maze (8 arm)	Spatial working memory in a radial arm maze in a novel room for 1 trial	c-Fos, with HPC, subicular and limbic cortices examined	Reduced c-Fos counts were found in the PL, ACC, RSC, dHPC and the vHPC

**Abbreviations:** ACC = anterior cingulate cortex; ATN = anterior thalamic nuclei; BLA = basolateral amygdala; CA1 = area CA1 of the hippocampus; CPu = caudate putamen; CREB = c-AMP response element binding protein; d = dorsal; DG = dentate gyrus; dHPC = dorsal hippocampus; dSub = dorsal subiculum; Ento = entorhinal cortex; GAP-43 = growth associated protein 43; HPC = hippocampus; IL = infralimbic cortex; l = lateral; LA = lateral amygdala; LD = laterodorsal thalamic nucleus; pCREB = phosphorylated c-AMP response element binding protein; PH = parahippocampal cortex; PL = prelimbic cortex; Postrh = postrhinal cortex; post-sub = post-subiculum; PRC = perirhinal cortex; pre-sub = pre-subiculum; Pt = parietal cortex; RAM = radial arm maze; Rdg = dysgranular retrosplenial cortex; Rga = granular a retrosplenial cortex; Rgb = granular b retrosplenial cortex; RSC = retrosplenial cortex; Sub = subiculum; sup = superficial; v = ventral; vHPC = ventral hippocampus.

Although only examined following ATN lesions, earlier intervals between lesion surgery and sacrifice were associated with a restriction of IEG changes to the superficial layers (layers II and upper III) of the Rgb and Rga regions (Jenkins et al., 2004; Poirier & Aggleton, 2009). As the interval between surgery and sacrifice increases, IEG activation appears to extend into the deep laminae of the Rgb and Rga, with the Rdg region also showing reduced IEG counts (Jenkins et al., 2004; Poirier & Aggleton, 2009).

Additional functional biomarkers such as c-AMP response element binding protein (CREB) and phosphorylated CREB (pCREB) have been examined in the retrosplenial cortex and hippocampus following ATN lesions (Dupire et al., 2013; Dumont et al., 2012). pCREB was considered of particular interest because of its role in neural plasticity, it is also involved in the regulation of IEG expression. In their study Dumont et al., (2012) found that ATN lesions only reduced pCREB in the Rgb, whereas Dupire et al., (2013) instead found a hypoactivation in the amygdala, Rgb and CA1. These difference might reflect different behavioural tasks used between studies.

Using a different approach, Harland et al., (2014) found that ATN lesions resulted in pronounced reductions of dendritic spine densities in hippocampal CA1 neurons and retrosplenial Rgb neurons. These microstructural changes are indicative of altered synaptic plasticity, and are most notable in the CA1 region because it is one of the primary sources of hippocampal outputs, and has no direct connection to the ATN (Harland et al., 2014).

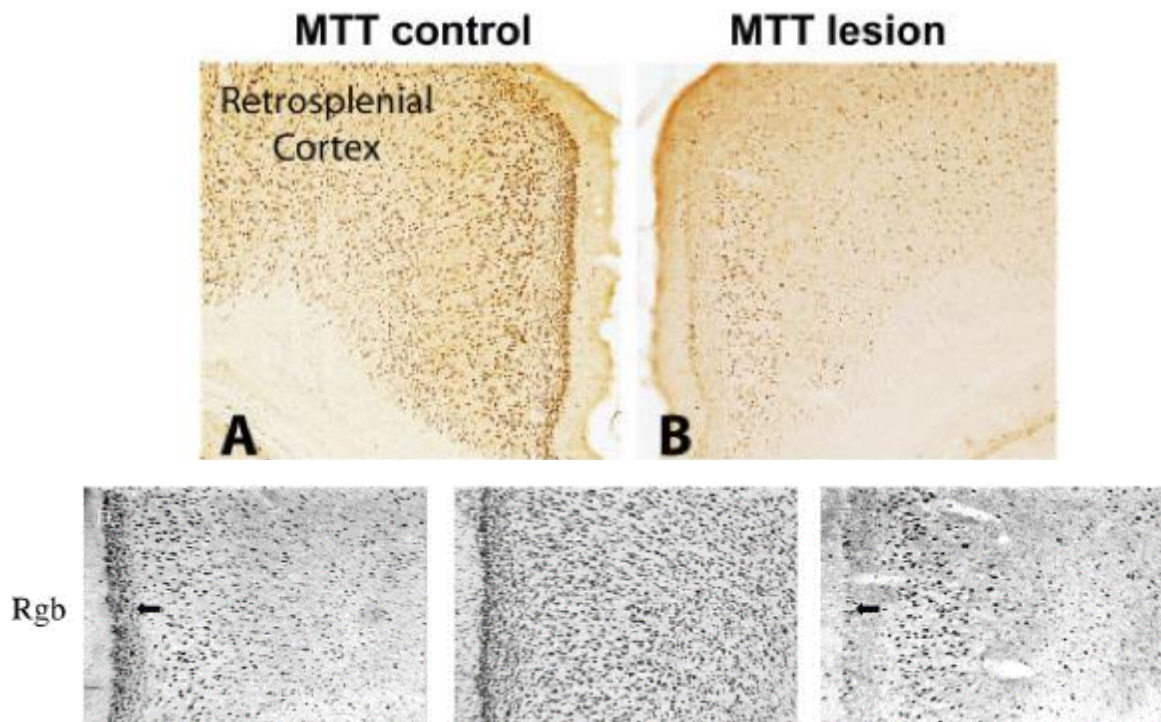


Figure 4.2: C-fos expression in the retrosplenial cortex following MTT lesions (top panel, adapted from Vann & Albasser 2009) and c-fos expression following ATN lesions (bottom panel, adapted from Poirier & Aggleton 2009)

Recently, Frizzarti et al., (2016), compared the effects of MTT lesions on zif268 and the metabolic marker cytochrome oxidase in the retrosplenial cortex and hippocampus to determine whether previously observed changes differed to the effects on c-Fos in these regions. In this study rats were run on a forced arm choice version of the RAM to drive the expression of the neural activity markers. MTT lesions decreased levels of both activity markers in the superficial and deep layers of the retrosplenial cortex in both its granular and dysgranular regions. Despite finding severe spatial working deficits in the T-maze, MTT lesions did not reduce zif268 staining in the hippocampus, unlike some but not all ATN studies find hippocampal hypoactivation of c-Fos (Loukavenko et al, 2016). No changes in cytochrome oxidase were found in the hippocampus (Frizzarti et al., 2016). The authors suggested that these findings are consistent with MTT lesions providing important, indirect inputs for normal RSC function. By contrast, albeit in separate studies, bilateral ATN lesions were found to reduce Cox activity only in the superficial lamina of the RSC granular b cortex (Mendez-Lopez et al., 2013).

Levels of neural activity markers are generally increased by introducing animals to stimuli prior to sacrifice to reduce the possibility of floor effects. This typically includes some form of novelty such as a new cage, new testing or holding room, or novel test cues (Jenkins et al 2002a,b; Jenkins 2004, Poirier et al., 2008; Poirier & Aggleton, 2009; Dumont et al., 2012). One task that has often been used prior to sacrifice is forced runs in a RAM (Jenkins et al., 2002a,b; Vann & Albasser, 2009; Dumont et al., 2012; Frizzarti et al., 2016). In this task entry to each arm is controlled by the experimenter so that lesion and control animals can be matched for motor responses and number of rewards received (Frizzarti et al., 2016). It is difficult to compare neural activity markers across studies as length of time since lesion surgery and subtle differences in behavioural tasks can all alter levels of expression. Therefore, one empirical study in the current thesis directly compared the impact of MTT and ATN lesions on *zif268* expression in the RSC and HPC within the same study. In addition, *zif268* expression was driven using a mid-trial maze rotation task in the RAM but with a reduced total number of arm entries to minimise spatial memory differences between rats. This task is more sensitive to RSC dysfunction than the standard task and was used in an attempt to ensure any changes in *zif268* expression were relevant to the behavioural deficit. The anomalous effects of MB lesions on object-place memory reported in the literature is examined in the context of MTT lesions and a behavioural procedure to address possible interpretations of performance in this task. A subsequent experimental study then examined the influence of MTT lesions on rhythmic oscillatory within key structures of the extended hippocampal system as well as the long-range interactions across these structures that may be necessary to support episodic memory function.

## Chapter 5

# Rhythmic oscillatory activity within the extended hippocampal circuit

This chapter reviews the literature providing evidence that rhythmic oscillatory activity supports the interactions necessary for mnemonic processes within the extended hippocampal system. Furthermore a case is made that impaired memory following lesions to the diencephalon may in part reflect altered rhythmicity between regions such as the hippocampus and prefrontal cortex. The following section discusses evidence from both human and animal studies suggesting that rhythmic oscillatory activity is involved in mnemonic processing. Theta and gamma activity have been consistently associated with successful memory performance in both humans and animals. Abnormal increases in low frequency oscillations has been associated with neurological diseases and has been shown to predict patients who will progress from MCI to dementia. There is also strong evidence for theta rhythmic activity within the diencephalon, but its functional role in mnemonic processes has yet to be determined.

As discussed in chapters 2, 3 and 4, both clinical and animal evidence has suggested that episodic memory is supported by an extended neural circuit connecting the hippocampus, diencephalon and limbic cortex (Aggleton & Brown, 1999; Child & Benarroch, 2013; Aggleton, 2014; Dillingham et al., 2015; Darlymple-Alford et al., 2015). Although the mnemonic role of individual structures has been a primary focus there is a need to recognise the system as an integrated neural circuit. Oscillatory activity is present in the brain, generated by the cumulative electrical activity of local neurons, and may present a means of information processing across interconnected structures (Buzsaki et al., 2011). Therefore, investigating changes to wide spread oscillatory interactions in cases of memory impairment may provide a more complete understanding of the underlying mechanisms of memory. In the future such evidence may also provide avenues for therapeutic interventions that target the system as a whole rather than as individual components.

### 5.1. Oscillatory activity in the brain

Neural oscillations of the extracellular field potential are generated by the rhythmic activation and inhibition of neural assemblies, and cumulatively these give rise to rhythmic activity in the brain (Fell & Axmacher, 2011; Buszaki et al., 2011). The frequency of these oscillations can range from relatively slow activity, with oscillation periods of several seconds, to fast activity in which one cycle lasts only a few milliseconds (Colgin, 2011). The partitioning of oscillatory activity into frequency bands can be somewhat variable across species, but here they are defined as: delta (1-4 hz), theta (4-8hz in humans, 4-12hz in rodents; in humans 8-12hz is more commonly described as alpha), beta (12-30hz), low gamma (30-48hz) and high gamma (52-100hz). Synchronisation of this rhythmic neural activity across structures is referred to as coherence (figure 5.1) and is proposed to be indicative of the functional interactions between brain regions necessary for complex cognitive functions such as memory (Fell & Axmacher, 2011; Colgin, 2013). For example, slow wave rhythmic activity such as theta, is capable of synchronising neural ensembles located distally in the brain. By contrast, fast oscillations such as gamma are thought to play a crucial role in local processing, and represent discrete information units (Jensen & Colgin, 2007; Colgin, 2013). For this reason it is postulated that interactions between theta and gamma can form a mechanism that encodes and temporally orders memory representations through a process known as cross frequency coupling (Jensen & Colgin, 2007; Nyhus & Curran, 2010). There is extensive research supporting a role for both theta and gamma rhythmicity and their interactions in human mnemonic processing. In the subsequent section a selection of relevant studies from the literature are provided to give a broad overview of theta and gamma involvement in memory.

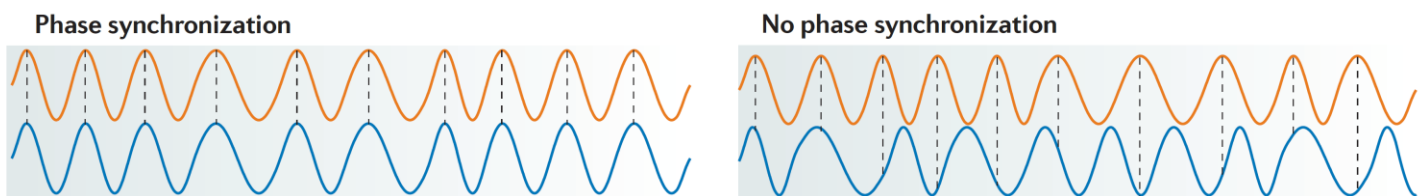


Figure 5.1: Diagrammatic representation of phase synchronisation or coherence (left) and no phase synchronisation (right) between oscillations. Adapted from Fell & Axmacher (2011)

## 5.2. Rhythmic oscillatory activity and memory in humans

The bulk of the available research investigating the electrophysiological correlates of episodic memory processing in humans has employed word recall/ recognition paradigms and recorded electroencephalograms (EEG) from scalp electrodes (Nyhus & Curran, 2010). Such studies have consistently reported that an increase in cortical theta phase synchronisation in healthy controls, and cortical-hippocampal theta coupling in epileptic patients, was predictive of successful memory processing (Colgin, 2013; Kilmesch et al., 1997; Weiss et al., 2000; Weiss & Rappelsberger, 2000; Mölle et al., 2002; Fell et al., 2003; Mormann et al., 2005). Research has also explored the involvement of theta-gamma interactions in the encoding and ordering of episodic memory. For example, the study by Mormann et al. (2005) explored interactions between theta and gamma oscillatory activity in the medial temporal lobe during a continuous word recognition paradigm in patients with epilepsy. The authors found that the perirhinal cortex and hippocampus relied on gamma oscillations during memory encoding and retrieval, whereas theta rhythmicity appeared to facilitate information transfer between these regions during the memory task (Mormann et al., 2005). Similarly, an earlier study reported that gamma rhythms appeared to hold memory representations whilst theta propagated these throughout the medial temporal lobe during a word recall task (Fell et al., 2003).

More recently, Heusser et al. (2016) provided a more explicit investigation of theta-gamma interactions by examining cross-frequency coupling during an episodic sequence memory task. Specifically this involved determining the degree to which the amplitude of gamma activity aligned with the underlying phase of a theta oscillation (figure 5.2e). Consistent with a critical role for cross frequency coupling, items in a sequence were found to be represented by spikes in gamma power along distinct phases of a theta oscillation and this segregation was related to successful temporal order memory (Heusser et al., 2016). Aside from its interactions with theta, studies have also shown that gamma oscillations may also play an independent role in memory function. Both cortical and intracranial recordings from patients with epilepsy suggest different roles for gamma within different neural regions. For example, frontal gamma is associated with attentional processing, hippocampal gamma with encoding and gamma activity in the temporal lobe with semantic elaboration, all processes necessary for successful mnemonic processing (Sederberg et al., 2003; 2007a; 2007b). In



addition to the considerable clinical evidence, animal studies have reinforced the critical role of theta and gamma rhythmicity in learning and memory processes.

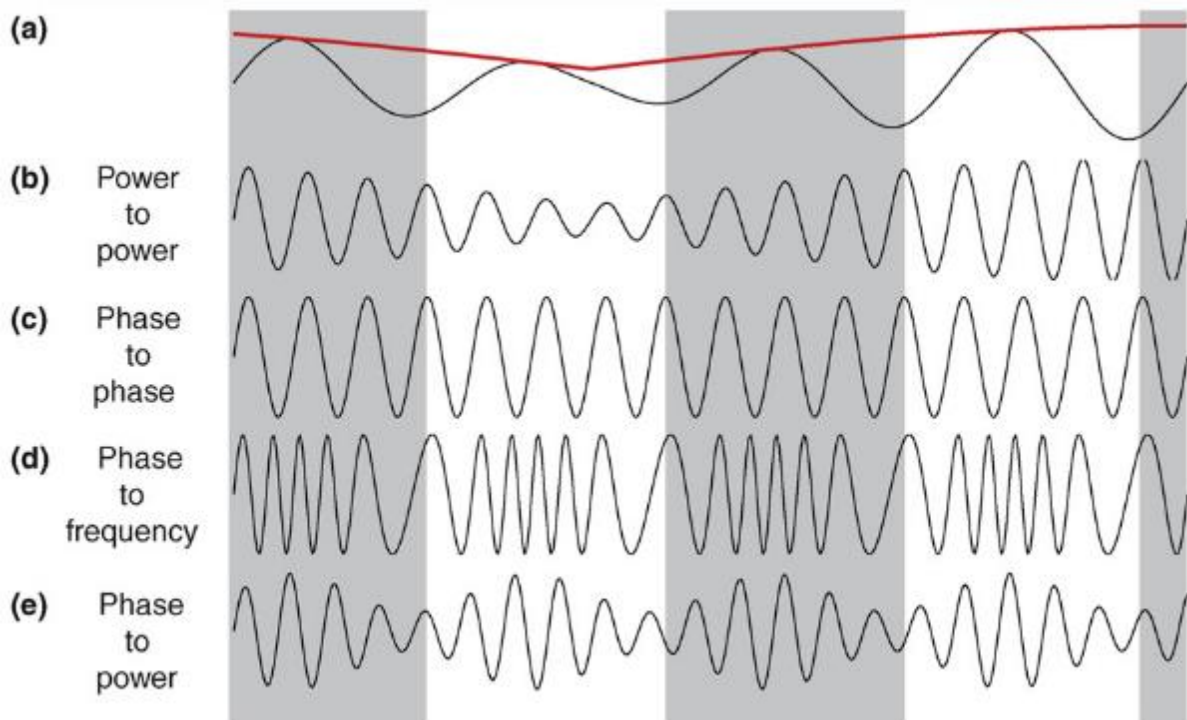


Figure 5.2: An example of the different principles of cross-frequency interactions, from Jensen & Colgin (2007). (a) Although frequency remains relatively constant, the power (red line) of the signal fluctuates. (b) The fluctuation in power of the faster oscillations e.g. gamma are correlated with power changes in the lower band e.g. theta. (c) Within each low frequency phase there are four high frequency phases. (d) The frequency of the fast oscillation is modulated by the phase of the slower oscillation. (e) The power of the fast oscillation is modulated by the phase of the slower oscillation.

### 5.3. Rhythmic oscillatory activity and memory in animals

Rodent studies have recapitulated the importance of theta and gamma oscillatory activity in successful memory processing. This literature has focused heavily on the hippocampal formation and prefrontal cortex, with little consideration given thus far to rhythmic activity within the diencephalon. Because of its ubiquity throughout the hippocampal formation, theta activity has received the greatest attention in rodents (Buzsaki, 2002). Compelling evidence directly implicating hippocampal theta in mnemonic processes was provided by McNaughton et al. (2006). In this study, inactivation of the medial septum was used to abolish



hippocampal theta. This resulted in severely impaired spatial memory in the water maze. However, when hippocampal theta was reinstated via a bypass circuit using data from the supramammillary nucleus, memory performance returned towards baseline levels (McNaughton et al., 2006).

There is also considerable evidence that coupling of rhythmic activity between the hippocampus and prefrontal cortex is fundamental to the acquisition and maintenance of spatial memories in rodents (Colgin, 2011, 2013). These studies consistently report increased phase locking between hippocampal theta rhythm and phasic firing of neurons within the medial prefrontal cortex during critical decision points in working memory tasks (Jones & Wilson, 2005; Hyman et al., 2010; O'Neill et al., 2013). For example, Hyman et al. (2010) found that 94% of theta-modulated neurons in the medial prefrontal cortex became phase-locked to hippocampal theta during correct trials, but not incorrect trials, in a delayed non-matching to place task in an operant chamber. Furthermore, the importance of HPC-PFC interactions does not appear to be restricted to working memory tasks, but is also involved when rats form object-place paired associations. Kim et al. (2011) found increased phase-locking between neural spiking in the medial prefrontal cortex and hippocampal area CA1 theta rhythm during correct object-place association. Moreover, maximum CA1-PFC theta coherence was observed during the decision point in this task, suggesting that coherence may play a crucial role during epochs of high cognitive demand. Thus far, however, little attention has been given to the rhythmic contribution of other structures within the extended neural circuit, particularly the ATN. There is also no evidence to date as to how diencephalic pathology influences HPC-PFC interactions. Recent human evidence has, however, suggested a critical role for ATN-neocortical interactions during memory encoding (Sweeney-Reed et al., 2014). In this study, Sweeney-Reed et al. (2014) found that increased theta coherence between the neocortex and ATN, as well as neocortical theta- ATN gamma cross-frequency coupling predicted successful recall at a later time point (Sweeney-Reed et al., 2014).

Additional work using rodent models has reinforced the importance of theta-gamma interactions for successful memory processes. For example, Tort et al. (2009) found that as performance accuracy increased on a paired-association spatial memory task, so too did the strength of theta-gamma coupling in hippocampal CA3, and this increased coupling was

maintained across training. Another means of examining theta-gamma interactions is to measure the degree to which an increase in amplitude of one frequency band is paralleled by an increase in amplitude of the other, also known as co-modulation (figure 5.2b). For example, an increase in theta power would be paralleled by an increase in gamma power, which is indicative of the simultaneous recruitment of both oscillatory mechanisms (Lisman & Buzsaki, 2008). Theta-gamma co-modulation within the hippocampus was shown to predict performance on a single-trial spatial memory task (Shirvalkar et al., 2010). In this study co-modulation was strong during successful memory retrieval and weak when memory failed or during a sample trial when there was no memory to be retrieved (Shirvalkar et al., 2010).

The aforementioned evidence provides a clear role for theta and gamma rhythmicity in mnemonic processing in intact neural systems. However, substantial insight into memory might also be gained by examining the emergence of electrophysiological aberrations present in cases of neurodegenerative and neuropsychiatric diseases associated with impaired memory in humans. It seems likely that any such oscillatory aberrations would contribute to the memory deficits that characterise such diseases.

#### 5.4. Evidence of aberrant rhythmic activity in neurological disease

Aberrant rhythmic activity has been associated with various neurological diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and schizophrenia, and likely contributes to the cognitive impairments associated with these conditions (Basar et al., 2016; Palop & Mucke, 2016; Senkowski & Gallinat, 2015; Nimrich et al., 2015). These changes commonly manifest as an abnormal increase in low frequency wave forms as a result of slowed rhythmic activity. Disruption of faster gamma oscillations is also consistently observed (Nimrich et al., 2015). Abnormal changes in the strength, occurrence and coherence of rhythmic activity in neurodegenerative diseases have been proposed to reflect the loss of neuromodulatory activity and specialised interneurons, which both contribute to oscillatory states. The loss of these mechanisms impacts the recruitment of networks of neurons at the specific frequencies necessary for highly complex cognitive functions such as memory (Nimmerich et al., 2015). For example, abnormal increases in theta rhythmicity have been observed in patients with AD, PD and schizophrenia, with more variable changes found in other frequency bands (Hermann & Demiralp, 2005; Nimrich et al., 2015; Cozac et al., 2016; He et al., 2016; Charurvedi et al., 2017). Critically, pathology within the diencephalon

has been associated with the cognitive impairments present in all of these conditions suggesting a possible link between diencephalic damage and changes to rhythmicity (Braak & Braak, 1991a,b; Delacourte et al., 1999; Young et al., 2000; Rub et al., 2002; Aggleton et al., 2016). Cortical EEG recordings from patients with AD have revealed altered rhythmic oscillatory activity over a wide range of frequencies. However, a shift from fast (alpha and beta) to slow (delta and theta) waves during spontaneous recording is consistently reported (figure 5.3, Nimmrich et al., 2015). For example, increased theta power and decreased alpha power at temporal and parietal sites were found to be the best predictors of which patients with mild cognitive impairment (MCI) would progress to AD over a 21 month period (Jelic et al. 2000). In a more recent study progression to AD over 2 years could be accurately predicted at baseline by lower cortical alpha activity and desynchronized alpha activity above the posterior cingulate during memory encoding (Prieto del Val et al., 2016). Studies have also reported global reductions in gamma power, which are thought to be indicative of pathological activity in the cortex as the result of reduced cholinergic input (Hermann & Demiralp, 2005; Nimmrich et al., 2015).

Like AD, significant aberrations in theta and gamma rhythmic activity has been observed in cognitively impaired patients with PD. For example, Cozac et al. (2016) found that an increase in global theta activity in conjunction with lower executive and working memory function at baseline could predict severe cognitive decline in PD over a three year period. Increased theta power in the temporal region has also been shown to differentiate between PD patients and healthy controls (Charuvedi et al., 2017), and PD patients with and without MCI (He et al., 2016). Like AD, patients with schizophrenia were also found to have reduced gamma power, but only during sensory encoding (Sun et al., 2013). Similarly, Chen et al. (2014) found that impaired working memory performance was associated with significantly lower gamma amplitude in the prefrontal cortex of patients with schizophrenia. Increased activity within the default mode network in lower frequency bands, such as theta has also been observed in schizophrenia (Burke et al., 2013). A recent report, also found that theta-gamma cross-frequency coupling in the frontal cortex was selectively impaired during correct choices in patients with schizophrenia during a working memory task (Barr et al., 2017).

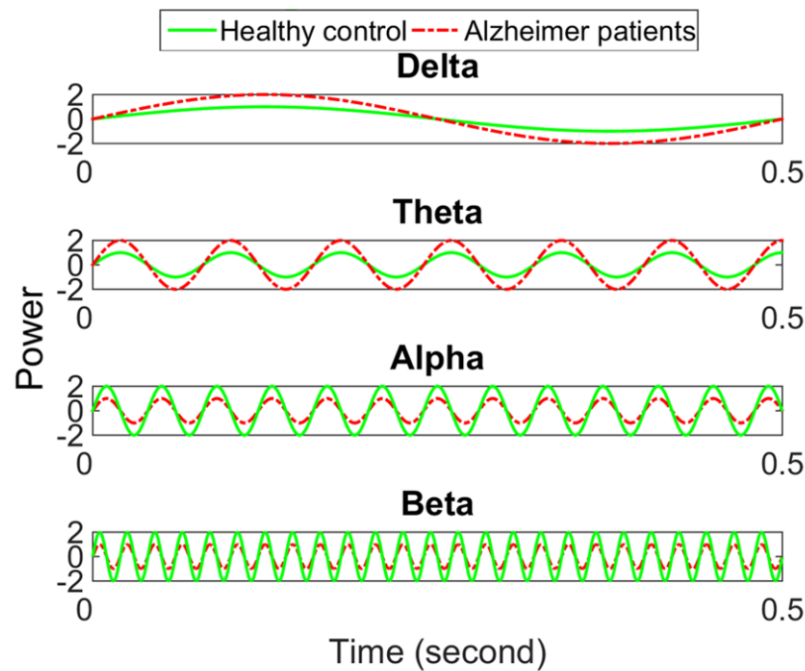


Figure 5.3: Schematic diagram demonstrating common EEG changes observed in Alzheimer's disease (From Nimmrich et al., 2015).

### 5.5. Evidence of aberrant rhythmic activity in animal models

Several methods have also been utilised to examine the behavioural and neurophysiological correlates of disease-related memory dysfunction in animals. For example, transgenic rodent models have allowed researchers to model critical neuropathological characteristics associated with neurodegenerative diseases, such as AD. These AD models, which focus on amyloid beta ( $A\beta$ ) and tau protein pathology, have been fundamental to our understanding of AD progression and symptomology. Severe memory impairments and dysfunctional oscillatory activity have been consistently reported across these AD mouse models. In line with clinical findings, transgenic mice over-producing  $A\beta$  showed a significant slowing of hippocampal theta power and frequency in mice (Scott et al, 2012). Furthermore in another  $A\beta$ -overexpressing mouse model, increased delta and theta power occurred at pre-plaque stages (Jyoti et al., 2010). Reductions in gamma oscillations have also been observed in transgenic mouse models of AD. In one example, Verret et al. (2012) found that mice with upregulated amyloid precursor protein, exhibited a loss of cortical gamma oscillations, which was associated with profound spatial memory deficits in the water maze. In another study,

using an amyloid-beta AD mouse model, substantial decreases in theta-fast gamma cross-frequency coupling were found in the subiculum prior to the occurrence of amyloid beta pathology (Goutagny et al., 2013). The evidence from AD mouse models suggests that electrophysiological alterations may be observed in the prodromal stages of the disease, and may contribute to the cognitive decline found in AD. Furthermore, in a maternal immune activation model of schizophrenia, HPC-mPFC coherence was observed to be profoundly reduced across the frequency spectrum (Dickerson et al., 2010). The aforementioned research indicates that aberrant rhythmic oscillatory activity, especially within the theta and gamma bands, may play a critical role in the cognitive deficits associated with neurological diseases.

### 5.6. Rhythmic activity within the extended hippocampal circuit

Studies examining the source of hippocampal theta rhythmicity have strongly implicated the medial septum/vertical limb of the diagonal band nucleus (MS/DBv) because these regions possess rhythmically discharging cells that fire synchronously with theta (Vertes et al., 2004). Furthermore, stimulation of the MS/DBv has been shown to drive hippocampal theta, whilst permanent or temporary lesions of the MS/DBv or disruption of MS/DBv rhythmic activity eliminated theta activity in the hippocampus and parahippocampal regions (Vertes et al., 2004). Similarly, inactivation of the medial septum has been shown to both impair memory and diminish theta rhythmicity in the hippocampus and MB (Kirk et al., 1996; McNaughton et al., 2006). However, work by Kirk and McNaughton (1991) provided evidence that the supramammillary nucleus (SuM) also modulates theta activity. This finding received additional support from earlier MS/DBv retrograde tracing studies, which on re-evaluation had direct projections from the SuM to the MS/DBv (Vertes & McKenna, 2000). Subsequent investigation of the SuM by Kirk and McNaughton (1993), found that SUM cells fire rhythmically at theta frequency, and that inactivation of SUM activity in anaesthetised rats disrupted rhythmicity in the medial septum and, more importantly, suppressed hippocampal theta (Kirk & McNaughton, 1993; Thinschmidt et al., 1995). However, in awake rats, suppression of SUM theta oscillations, either chemically or with extensive lesions was shown only to reduce theta frequency rather than eliminating it per se (McNaughton et al., 1995; Sharp & Koester, 2008). This led to the proposal that the SUM codes the frequency of theta rhythm and that the medial septum determines the amplitude of the theta wave form (Kirk & McNaughton, 1993; Vertes et al., 2004). Currently, control of hippocampal theta appears to

rely on multiple modulatory structures, with the overarching coordinating input yet to be determined (Buzsaki, 2002).

Although the focus has been on the origin of hippocampal theta, the presence of theta throughout other structures of the extended circuit reinforces the concept of theta as critical coordinating rhythm throughout the system (Kirk & Mackay, 2003; Ketz et al., 2015). For example, theta oscillations have been found within the MB and the ATN. Cells within these two structures exhibit theta bursting activity in synchrony with hippocampal theta, suggesting a common source of rhythmic input (Kocsis & Vertes, 1994). However, unlike the hippocampus and entorhinal cortex, the ATN and MB in rodents do not contain any interneurons so are not capable of independently generating this rhythmic activity (Wang et al., 1999; Dillingham et al., 2015). Thus, theta activity within the ATN and MB must be driven by afferent structures. For the ATN this is likely from ascending inputs from the MB via the MTT or from descending inputs from the hippocampus via the fornix. Similarly, the MB also receive a hippocampal input via the fornix, as well as a unique brainstem input from the VTg (Vann & Nelson, 2015). Like the ATN and MB, theta activity is also observed in the VTg (Kocsis et al., 2001). However, the VTg is capable of independently generating theta and the firing of VTg cells appears to precede hippocampal theta by 1-2 seconds, suggesting that the VTg may be a pontine hippocampal theta generator (Bassat & Poindessous-Jazat, 2001). However, it is not yet clear whether rhythmic activity within these regions is capable of modulating hippocampal activity.

Whilst the presence of theta oscillatory activity within the diencephalon is well-established, the source, function and direction of information flow of this rhythmic activity is unclear. Currently there are two scenarios both of which centre around which of the two primary MB inputs exerts the most influence over theta activity. One view has emphasised the critical role of the septo-hippocampal axis in driving theta rhythmicity throughout the diencephalon (Kocsis & Vertes, 1994; Kirk et al., 1996; Kirk & Mackay, 2003). The hippocampal formation is primarily connected to the diencephalon via the fornix, providing a direct route of rhythmic propagation from the medial septum. Evidence for this position has come from work showing that septal blockade eliminates theta activity in both the hippocampus and the MB (Kirk et al., 1996). Furthermore rhythmic bursting of MB theta cells was highly correlated with oscillatory theta activity in the hippocampus, especially area CA1 (Kocsis & Vertes, 1994). These two findings have been interpreted as evidence that MB

theta activity is driven by its septo-hippocampal inputs. An alternate hypothesis is that the VTg provides a unique brainstem theta input, via the mammillary peduncle, that converges with septo-hippocampal innervation in the MB before ascending to the ATN (Bassant & Poindessous-Jazat, 2001; Vann & Aggleton, 2004). As previously mentioned, the rhythmic theta bursting of VTg cells precedes hippocampal theta rhythm. Furthermore, severing the post-commissural fornix, the fibre tract providing hippocampal input to the MB, results in negligible behavioural effects (Vann et al., 2010; Vann, 2013), although its impact on MB and ATN rhythmicity has not been examined. Although not definitive, these findings raise questions as to the importance of the hippocampal input into the MB for both memory and theta propagation. Therefore it might be that the VTg can modulate hippocampal and neocortical rhythmic activity via the MB and ATN, forming a circuit, which projects back upon itself, providing a feedback loop.

This recurrent loop has been proposed to centre on the anteroventral and anteromedial components of the ATN and involve the medial MB and ventral tegmental nuclei of Gudden (Aggleton et al., 2010; Ketz et al., 2015). It is also of note that the lateral aspects of these same structures form a parallel head direction system (Vann & Aggleton, 2004). The defining feature of the structures within the head direction circuit is the presence of head direction cells, which are specifically tuned to fire when a rat faces a certain orientation (Taube & Muller, 1998). This system appears to be hierarchically organised with the information flow being driven by brainstem inputs in the dorsal tegmental nuclei of Gudden and ascending to cortical regions via the lateral MB, the anterodorsal nucleus of the thalamus and the hippocampal formation (Tsanov, 2015). Consistent with this proposal, lesions to the subcortical components of the head direction circuit impact the cortical head direction signal, whereas cortical lesions have minimal impact on the activity of head direction cells in subcortical structures (Dillingham et al., 2015).

Whilst these systems appear to function independently there is also evidence that these two signals, that is theta and head direction activity, may converge and integrate within the anteroventral nucleus, a key component of the theta circuit but which also contains a sub population of head direction cells (Tsanov, 2015). In fact, 39% of these AV head direction cells fire at theta rhythm, suggesting that the transfer of information propagated by head direction cells in the AV may be facilitated by theta (Tsanov et al., 2011b). This integration of information at the level of the AV reinforces the importance of diencephalic-hippocampal

interactions. The theta circuit has been strongly implicated in mnemonic processes but the aforementioned evidence suggests that there may also be overlap between theta activity and the head direction circuit. A greater understanding of the electrophysiological activity of these two circuits may demonstrate how interactions between them support memory function.

## 5.6. Diencephalic lesions and rhythmic oscillatory activity

It appears that pathological changes within an extended network, with diencephalic and hippocampal regions at its core, contributes to the profound memory impairments observed in neurodegenerative diseases such as AD (Aggleton et al., 2016). It is proposed here that lesion-induced memory deficits in part result from aberrant interactions between structures within the extended system. Here, the electrophysiological interactions between the hippocampus, PFC and retrosplenial cortex are of particular importance due to their consistent implication in spatial memory impairments and the presence of reciprocal interconnections (Aggleton et al., 2010; Preston & Eichenbaum, 2013). At present little is known about the impact of localised damage to the diencephalon on systemic rhythmic activity. Therefore when examining this, it is valuable to select behavioural tasks which drive memory processes, and inter-structural interactions within the circuit, thus enabling accurate characterisation of electrophysiological activity as it directly relates to behaviour. Tasks that may be appropriate and the subsequent electrophysiological changes that might be expected in the hippocampus and PFC are discussed below. As previously alluded to the ATN, MTT, MB and VTg are considered key subcortical components of the memory circuit, and so provide a focus for lesion studies (Aggleton & Nelson, 2015; Dillingham et al., 2015; Dalrymple-Alford et al., 2015). While accurate paradigms have been developed in humans to link encoding and retrieval with temporally-specific rhythmic activity, this has not been as well characterised in rodent research. Rather than selecting general memory tasks, a superior approach might be to select tasks that are sensitive to damage or dysfunction within the extended memory system. For example, tasks in the radial arm maze or conditional associative tasks involving odour-place or object-place associations, are beneficial as they are known to be dependent upon the hippocampus and prefrontal cortex (Floresco et al., 1997; Kesner & Ragozzino, 2003; Gilbert & Kesner, 2004). Selective lesions of the ATN, MTT, VTg, or MB, alongside electrophysiological analysis of interconnected regions within the



memory circuit may demonstrate the presence of electrophysiological aberrations that accompany profound behavioural deficits.

Therefore, an investigation of the impact of lesions to the ATN, MTT, VTg or MB on rhythmic interactions within the system during a delayed RAM task or conditional associative learning task would provide highly novel insight into the contribution of the diencephalon to rhythmic activity. Furthermore, special emphasis should be given to the hippocampus and PFC due to their primacy in encoding, consolidation and retrieval processes (Preston & Eichenbaum, 2013). Here, two suitable tasks and the predicted electrophysiological outcomes are described.

Rats with ATN, MB, MTT and VTg lesions are all impaired in the standard radial arm maze task (Byatt & Dalrymple-Alford, 1996; Aggleton, 2003; Mitchell & Dalrymple-Alford, 2006; Vann & Vann, 2013), so the consistency of these deficits make the RAM an ideal task for analysis. This task can also be manipulated, for example the addition of a delay following the first four arm choices increases task difficulty and may exacerbate the lesion deficit. The parameters of the delay RAM task allow the researcher to contrast a period of low cognitive load (first four arm choices, pre-delay) with a period of high cognitive load (following delay). The addition of beam breaks placed at critical points down each arm can be used to differentiate between the oscillatory activity directly associated with choice behaviour, when both encoding and retrieval mechanisms should be employed, such as the epoch before the rat begins running down the arm during early and later choices. Consequently, we would suggest using the delay RAM task to highlight the memory deficits associated with each lesion, drive neural interactions and extract relevant data as it directly pertains to behaviour. In line with previous work, it would be expected that sham rats would have a peak in theta coherence between the HPC-PFC during an arm selection (Jones & Wilson, 2005; Hyman et al., 2011; O'Neill et al., 2013). If the VTg is a “pontine theta generator” contributing to both diencephalic and hippocampal rhythmic activity (Bassant & Poindessous-Jazat, 2001), lesions to the VTg, MB, MTT and ATN, which would all disrupt this input's influence on upstream cortical structures, would be expected to result in a similar reductions in hippocampal theta power and HPC-PFC theta coherence. However, ATN lesions may have a more profound effect on rhythmic activity in the PFC and RSC given their dense reciprocal connections to these regions (Jankowski et al., 2013; Dalrymple-Alford et al., 2015). Alternately, if the descending septo-hippocampal projections are primarily driving rhythmic

theta activity within the diencephalon, rather than the VTg, lesions to the MB, MTT and ATN would be expected to disrupt theta coherence between the HPC and PFC. In this alternate scenario VTg lesions could be expected to result in mild or even negligible change in theta coherence between the HPC and PFC.

As indicated, an important aspect of episodic memory is the formation of associations between arbitrary stimuli. The hippocampus plays an important role in this process, although likely only when the association involves a temporal or spatial context (Gilbert & Kesner, 2004). Whilst the hippocampus plays a primary role in association formation, cortical interactions are required for maintaining and consolidating these associations (Kesner & Ragozzino, 2003). The contribution of independent structures to association formation can be tested using tasks that require the pairing of reward with the association between a spatial location and a particular item such as an object or odour (Gilbert & Kesner, 2002). As this task requires an extended period of learning before control rats reach a behavioural asymptote in performance, researchers are able to obtain large quantities of data over successive days. As a result, data can be broken down into specific epochs, and comparisons can be made between patterns of electrophysiological activity within and between subjects during initial acquisition and later stable performance. It is noteworthy that studies in rats have shown that lesions to the ATN, but not the MB, impaired acquisition of object-place associations (Sziklas et al., 1996; Sziklas & Petrides, 1999). This finding represents an anomaly within the literature so this paradigm was first re-examined by the current thesis (see chapter 7). It is possible that in Sziklas et al. (1996) MB lesion rats were able to adopt directional strategies to solve this task, because recent evidence has suggested that rats with MTT lesions are impaired on a spontaneous object-in-place task and place discrimination when directional information is irrelevant (Nelson & Vann, 2014).

However, the effects of MB lesions and VTg lesions also require (re)examination in paired-associate learning tasks. Assuming a deficit is found, we would expect that MTT, MB, VTg and ATN lesions would reduce theta coherence between the HPC and PFC especially when rats are making an object choice. Moreover, due to the afore-mentioned extended acquisition period required for bi-conditional paired-association tasks, changes in hippocampal-prefrontal cortex coherence and power can be examined over time. Control rats would be expected to demonstrate increases in theta coherence between the HPC –PFC with improved performance. Similarly, although to a lesser extent than control rats, we would

expect improved HPC-PFC theta coherence to be observed across lesion groups with extended training, with the possible exception of ATN lesions because a previous study showed no evidence of discrimination in rats with ATN lesions even after 500 trials (Sziklas et al., 1999). The aforementioned research may be further expanded through the comparison of the relative coherence increases across lesion type, as this may be indicative of the contribution made by each of the diencephalic and brainstem structures of interest.

Carefully selecting tasks that drive activity in the hippocampus and PFC, but that also rely on the functional integrity of other components of the extended hippocampal circuit, would enable researchers to elucidate the contributions made by independent structures to memory. Furthermore, focussing on changes in coherence between the hippocampus and PFC may help to determine how loss of these diencephalic inputs impair the critical interactions between these two primary regions, and in doing so, contribute to the substantial memory deficits that arise.

## 5.7. Rhythmic activity and recovery of function

Not only can a greater understanding of rhythmic oscillatory activity within the extended hippocampal system provide a more complete view of the neural underpinnings of memory, but it may also give insight into previously unexplained instances of spontaneous recovery. One interesting aspect of the alcoholic Korsakoff's syndrome (KS) is that approximately 75% of patients improve with abstinence (Kopelman et al., 2009). Critically, these cases of recovery occur despite considerable permanent neuropathology within the ATN and MB, as well as in frontal cortical regions (Harding et al, 2000; Kopelman, 2014; Aggleton, 2014). Therefore, the recovery observed in these cases suggests a degree of compensation must be occurring elsewhere in the memory circuit (Dalrymple-Alford et al., 2015). Further evidence of recovery in KS has been provided by the pyridoxamine-induced thiamine deficiency (PTD) rat model of KS, which has been consistently used to examine the KS-associated pathological changes (Savage et al., 2012). This model consistently produces permanent lesions in the diencephalon, along with functional abnormalities in the hippocampus, prefrontal and retrosplenial cortices. Like patients with KS, there is also evidence of functional recovery in rats with PTD after thiamine replacement (Savage et al., 2012). Moreover, these improvements appear to be occurring in regions associated with functional abnormalities in

the disease state. For example, in the PTD model a loss of cholinergic innervation is observed in the hippocampus, frontal and retrosplenial cortices, and this is thought to contribute to the spatial memory deficits. However, selectively increasing acetylcholine levels in the medial septum, hippocampus and frontal cortex can ameliorate these behavioural deficits (Savage et al., 2012).

In line, with the proposed role of rhythmic activity in memory function, is the observation that cholinergic signalling is proposed to play a critical role in the generation of oscillatory states throughout the CNS (Drever et al., 2011). Therefore, aberrations in acetylcholine activation, especially within the hippocampus, would likely disrupt circuit wide rhythmicity and contribute to the memory impairments. This dysfunctional activity is particularly relevant due to the considerable evidence that PFC-hippocampal interactions are critical for memory function (Colgin, 2011). Therefore, it may be that the recovery of function observed following acetylcholine intervention reflects improved oscillatory interactions.

Enhanced rhythmic activity within the extended hippocampal circuit could also explain the recovery of function observed following environmental enrichment in rats with lesions to the diencephalon (Loukavenko et al., 2007; 2016; Wolff et al., 2008; Harland et al., 2014). As previously mentioned, ATN lesions cause profound memory deficits in rats, but subsequently exposing these rats to enriched environments has been shown to significantly improve spatial memory performance. The mechanisms underlying this recovery of function are still far from well understood, although it has been suggested that alterations in hippocampal CA1 may have crucial role (Harland et al., 2014). It may be that environmental enrichment ameliorates memory performance via improved rhythmic oscillatory activity and inter-structural interactions as initial evidence suggests that environmental enrichment improves theta coherence between the PFC and hippocampal CA1 in both ATN and sham rats (Ulrich et al., in prep).

Improved oscillatory activity and restoration of theta may also explain an anomaly within the ATN literature (Beracochea et al., 1989). In this study, ATN lesions were made unilaterally in two separate surgeries one week apart because of excessive mortality when bilateral lesions were given. These staged lesions did not impair spatial working memory in the radial arm maze, which is inconsistent with the severe ATN-associated memory deficits

found in this task in subsequent work (Aggleton & Nelson, 2015). Here we suggest that the lack of behavioural deficit found by Beracoche et al. (1989) might be a result of adaptation or compensation allowed to occur because of the more gradual nature of the lesion. For example, rhythmic interactions between the HPC, PFC and RSP may have strengthened after initial damage enabling the system to compensate prior to the subsequent bilateral ATN loss.

## 5.8. Concluding remarks

Both clinical and animal evidence highlight the need to look beyond the hippocampus in order to gain a more balanced understanding of the neural substrates underlying memory function. There is a plethora of evidence suggesting that damage to components of the diencephalon, especially the ATN-MTT-MB axis, consistently results in profound memory impairments. It is also clear that damage to the diencephalon produces functional alterations throughout the extended hippocampal system, including reduced IEG and CO activity, and fewer dendritic spine counts, which seem likely to contribute to the behavioural deficits. The most profound of these changes are indicated by reductions in IEG activation in the retrosplenial cortex. Rat studies have revealed that the hippocampus and diencephalon function interdependently during spatial memory tasks. However, the exact nature of these interactions is not well-defined and greater insight into the underlying neural representation of memory may come from determining how damage to these structures impacts the interactions between the two primary memory regions, the PFC and hippocampus. In line with this, oscillatory activity within the hippocampus has been heavily implicated in memory, and it may be that rhythmic synchrony between the prefrontal cortex, diencephalon and the hippocampus may provide a mechanism of interaction during mnemonic processes. Evidence from human cases of cognitive dysfunction and animal models has suggested that aberrant oscillatory activity across the extended memory circuit likely contributes to the memory deficits observed. However there is little research exploring the impact of selective damage on individual components within the system on circuit-wide rhythmicity and how this applies to memory dysfunction. Here we recommend selecting behavioural tasks that are relevant to the structure of interest, but that also drive interactions between the hippocampus and PFC. Implementing behavioural tasks in such a way allows researchers to examine the impact of diencephalic lesions on electrophysiology both within and between the hippocampus and PFC.

Gaining a better understanding of disrupted network oscillations may provide directions for targeted therapeutic intervention aimed at restoring mnemonic functioning in cases of neurodegenerative disease, such as AD, or following more localised damage, such as that found in thalamic stroke. In fact, it may be that aberrant electrophysiological activity might in part explain some of the cases of recovery observed following interventions such as environmental enrichment or in instances of spontaneous recovery, for example in Korsakoff's syndrome patients.

In the subsequent chapters (6-8) the behavioural, neural and electrophysiological impact of MTT lesions are investigated across three empirical experiments, followed by a general discussion in chapter 9. In Chapter 6 a direct comparison between MTT and ATN lesions is made to provide insight into two different perspectives regarding the critical site for diencephalic amnesia. Chapter 7 extends previous MTT lesion findings beyond standard spatial working memory tasks by testing rats on an object-place paired associate task, which is particularly relevant to human memory and clinical amnesia. Chapter 8 examines the impact of MTT lesions on rhythmic oscillatory activity both within and between critical structures within the extended hippocampal circuit during spatial working memory performance. In addition, parameters for electrical stimulation of the ATN were explored that could be used to enhance spatial memory performance in rats with MTT lesions.

# Chapter 6

## MTT and ATN comparison

### 6.1. Introduction

Prior chapters demonstrated that both the anterior thalamus nucleus (ATN) and the mammillothalamic tract (MTT), the unidirectional afferent path from the mammillary bodies (MB) to the ATN, are two critical structures within an extended hippocampal-diencephalic circuit associated with episodic memory (Child and Bennarroch 2013; Aggleton 2014; Dalrymple-Alford et al., 2015). Injury to the MTT is the most consistent predictor of an amnesic syndrome following thalamic infarction (Van der Werf et al 2000, 2003; Carlesimo et al 2011; Carlesimo et al., 2014). ATN pathology may be the critical point of difference between Korsakoff amnesic patients and patients with alcoholic Wernicke's encephalopathy but no amnesic syndrome (Kopelman 2014; Harding et al. 2000). Other clinical evidence suggests a significant influence of MB pathology in developmental amnesia whose memory loss was previously interpreted solely on the basis of their hippocampal pathology (Dzieciol et al. (2017) and MB degeneration is critically associated with memory loss after fornix injury after brain surgery (Tsivilis et al, 2008). Uncertainty regarding the boundaries of diencephalic pathology and influence of pathology elsewhere in clinical cases of diencephalic amnesia limits firm conclusions regarding the specific contributions of the MTT and ATN to memory. Nonetheless, there is increasing evidence that the impact MTT injury reflects a more prominent influence of the brainstem on the integrity of ATN function than previously recognised (Dillingham et al., 2015; Vann and Nelson, 2015), so equivalent deficits after MTT and ATN injury might be expected to be the rule rather than the exception.

While both anatomical and clinical evidence implicates a functional overlap between the MTT and the ATN with respect to episodic memory processes, to date the relative impact of injury to these structures has not been directly tested. Thus far, only separate experimental lesion studies have examined the effects of highly localised MTT and ATN lesions on memory. Comparing across studies, both MTT and ATN lesions have consistently produced spatial working memory deficits in the T-maze and radial arm maze (RAM) (Aggleton & Nelson, 2015, Dalrymple-Alford et al., 2015; Nelson and Vann 2014; Frizzarti et al., 2016),

although these effects are sometimes more transient with MTT lesions (Vann & Aggleton 2003; Vann 2013). The effects of MTT and ATN lesions appear, however, to be dissimilar on other spatial memory tasks. For example, MTT lesions in rats were reported to show weak and transient reference memory impairments in the standard water-maze in one study and did not impair geometric learning in a water-maze in another study (Winter et al. 2011, Vann 2013). By contrast, ATN lesions produce profound deficits in both of these tasks (Warburton et al., 1999; Wolff et al., 2008; Aggleton et al., 2009; Dumont et al., 2014). Nonetheless, it remains possible that some of the differences between the effects of MTT and ATN lesions are a result of minor procedural variations across studies.

One of the most interesting features of MTT and ATN lesions is that they both produce a dramatic loss of immediate early gene (IEG) neural activity markers in distal regions of the extended hippocampal memory circuit, despite ostensibly normal conventional histology (Aggleton, 2008; Nelson and Aggleton 2015; Dillingham et al., 2015). This apparent “covert functional pathology” suggests that both diencephalic lesions have a similar impact on the function of an extended memory system. The retrosplenial cortex appears to be particularly sensitive to these two diencephalic lesions because separate laboratories have consistently found substantial hypo-activation of c-Fos and zif268 IEGs in this cortical structure (Dillingham et al., 2015; Nelson and Aggleton 2015; Loukavenko et al., 2016; Frizzati et al., 2016). More variable changes have been observed in the hippocampus across ATN and MTT lesion studies, suggesting that the specific neural marker or the behavioural task used may explain these differences (Jenkins et al., 2002a,b; Poirier and Aggleton 2009; Dupire et al., 2013; Vann 2013, Loukavenko et al., 2016; Frizzati et al., 2016). Similarly, both ATN lesions and MTT lesions reduce the level of the metabolic marker cytochrome oxidase in the superficial lamina of the retrosplenial cortex, but MTT lesions reduce expression in the deep layers of this limbic cortex (Mendez-Lopez et al., 2013; Frizzati et al., 2016). Clearly, an understanding of the relative magnitude and specificity of the distal neural changes evident after MTT and ATN lesions would benefit from direct comparisons within the same study.

The current study directly compared the behavioural and neural effects of MTT lesions and ATN lesions in rats. We determined their relative impact on spatial working memory in the radial arm maze (RAM) and both spatial reference memory and spatial working memory in the water maze. Prior to sacrifice, we used a reconfigured room and



mid-trial RAM rotation as this procedure highlights the involvement of the retrosplenial cortex in spatial memory (Vann & Aggleton, 2002; Vann, Wilton, Muir, Aggleton, 2003; Pothuizen et al., 2008). We compared levels of zif268 in the retrosplenial cortex and hippocampus because zif268 expression is associated with spatial memory formation and long-term plasticity in the hippocampus whereas c-Fos is a more general measure of neural activation (Jones et al., 2001; Penke et al., 2014; Farina & Commins, 2016). While post-mortem clinical studies have not assessed IEG markers, they often report reduced neurons and volume in the MB in amnesic Korsakoff patients (Kopelman 2014; Harding et al., 2000); effects on the MB after MTT injury in stroke patients have not been reported. An additional aim therefore was to compare neuronal integrity in the MB after MTT and ATN lesions.

## 6.2. Materials and Method

### 6.2.1. Animals

Subjects were 55 male PVGc hooded rats bred in our facility and maintained in standard housing of three or four rats per opaque plastic cage (50 cm long x 30 cm wide x 23 cm). They were 8-10 months old and 320g to 430g at lesion surgery. Triplets of rats were randomly allocated to MTT lesion, ATN lesion or sham surgery groups using matched ranked performance across 12 days of preoperative working memory testing in an 8-arm radial arm maze (RAM). Behavioural testing was conducted during the lights off period (8am to 8pm). Individual housing was used for 7 - 12 days of recovery following surgery. Food and water were available ad libitum during surgery, recovery and initial behavioural tasks. Pre-surgery testing and later behavioural tasks required food restriction to attain 85% of their free feeding body weight, with water ad libitum. All procedures complied with the University of Canterbury animal ethics guidelines and were subject to AEC approval.

### 6.2.2. MTT surgery

Rats were anaesthetised ip with ketamine (80mg/kg) and domitor (0.35mg/kg) and placed in a stereotaxic device with atraumatic ear bars (Kopf, Tujunga, CA). Flat skull was used for MTT lesions, which were made using a Radionics TCZ radio frequency electrode (0.3 mm long, 0.25 mm diam exposed tip; RFG4-A, Radionics, Burlington, VT). One of four anterior-posterior (AP) coordinates relative to an individual rat's bregma to lambda (B-L) distance was used: from Bregma, -2.5 for B-L  $\geq$  6.4; -2.55 for B-L 6.5 to 6.8; -2.6 for B-L 6.9 to 7.2; -

2.65 for  $B-L < 7.2$ . The laterality was  $\pm 0.9$  from the midline and ventrality was  $-7.2$  from dura, with the electrode lowered vertically to the MTT coordinate, where the temperature of the tissue surrounding the electrode tip was raised slowly to  $63^{\circ}\text{C}$  and then maintained for 60 seconds. Sham rats received the same procedure except the electrode was lowered 1mm above the site and the temperature was not raised.

### 6.2.2. ATN surgery

For ATN surgeries the incisor bar was set at  $-7.5\text{mm}$  below the interaural line to minimise fornix damage. Two infusions per hemisphere were directed at the anteroventral nucleus (AV), at an upper and a lower site and one infusion per hemisphere was directed at the anteromedial nucleus (AM). As for MTT lesions, each surgery used one of four anterior-posterior coordinates relative to an individual rats B-L distance. For AV lesions the AP coordinates from bregma were:  $-2.5$  for  $B-L \leq 0.64$ ;  $-2.55$  for  $B-L 6.5$  to  $6.8$ ;  $-2.6$  for  $B-L 6.9-7.2$ ;  $-2.65$  for  $B-L \geq 7.3$ . The AV infusions were made at ventrality  $-5.63$  followed by  $-5.73$  from dura at  $\pm 1.52$  lateral to the midline. The AM infusion was placed  $0.1\text{mm}$  more anterior than the AV sites, with laterality  $\pm 1.20$  and ventrality of  $-5.86\text{ mm}$ . Lesions were made by infusing  $0.15\mu\text{l}$  per site of  $0.15\text{M}$  N-methyl-D-aspartate (NMDA; Sigma, Castle Hill, NSW) in  $0.1\text{M}$  phosphate buffer (pH 7.20) at a rate  $0.04\mu\text{l}$  per minute via a  $1\mu\text{L}$  Hamilton syringe (Reno, NV, USA) driven by a micro infusion pump (Stoelting, Wooddale, IL). The four AV sites were always lesioned before the two AM sites. Following infusion the needle was left in situ for a further 3 minutes per site for the neurotoxin to diffuse. Sham surgeries used an identical procedure except that the needle was lowered to  $1.5\text{mm}$  above the lesion sites and no material was infused.

### 6.2.3. Behavioural testing

#### 6.2.4. Radial arm maze task

Spatial working memory was tested using an 8-arm radial maze located in one corner of a large windowless room ( $4\text{m}$  by  $4.7\text{m}$ ). The maze was raised  $67.5\text{ cm}$  off the floor and consisted of a  $35\text{ cm}$  wide octagonal wooden hub painted black with 8 detachable aluminium arms ( $65\text{ cm}$  long by  $8.6\text{cm}$ ,  $3.9\text{ cm}$  high). Clear Perspex barriers ( $19\text{cm}$  x  $25\text{ cm}$ ) extended along each arm from the central hub to deter rats from jumping across arms. Entry to each arm was controlled by clear Perspex doors ( $9\text{cm}$  wide by  $25\text{ cm}$  high) which could be raised

singly or together by an overhead pulley system. At the far end of each arm was a wooden block 5×9×3 painted black with a recessed food well in the centre (3cm x 1cm) where chocolate pieces (0.1g) were placed. Inaccessible food rewards were also placed underneath the food well to provide constant odour cues.

#### 6.2.5. RAM habituation

Prior to radial arm maze habituation rats were food deprived down to 85% of their free feed weight, which took approximately 2 weeks. During this time each rat was handled for 5 minutes of per day and habituated to the chocolate drops (0.1g) that served as the reward for this task. Once the rats approached the 85% target they were habituated to the radial arm maze for 4 days, first in cage groups then singly.



Figure 6.1: Photograph depicting the spatial configuration of the room used for testing and the RAM used for this experiment.

#### 6.2.6. RAM procedure

Following habituation the rats received 12 consecutive days of testing in the standard working memory version of the radial arm maze. For this task all eight arms were baited only once and the most efficient strategy was for the rat to consume all the food rewards without revisiting an arm. Prior to starting the food wells at the end of the eight arms were baited with

two chocolate pellets each. The rat was then carefully placed in the central hub and following a ~10 second delay all eight arms were opened and the rat was allowed to make a choice. An arm choice was defined as both back legs down an arm no back tracking. Once the rat had entered an arm all the doors were then closed and the rat was confined to the arm for ~15 seconds while it ate the food reward. If the rat selected an arm it had entered previously it was considered an error and the rat was confined to the arm for ~15 seconds. After the ~15 second delay the arm door was raised and the rat was allowed back into the central hub, where it was held for ~10 seconds before all the doors were again opened and the rat made another arm selection. A trial concluded when, the rat had visited all 8 arms, 20 arm choices had been made or 10 minutes had elapsed. Following each trial the maze was rebaited for the next rat.

### 6.2.7. Water maze

#### 6.2.8. Apparatus

The water maze was constructed out of white rigid plastic and had an internal diameter of 180cm with a height of 45cm with an outer lip protruding 5cm (Figure 6.1). It was located off centre on the floor of a windowless room (4m by 4.7m). The water maze was filled to a height of 30cm with water that was  $21 \pm 2^\circ\text{C}$  made opaque by the addition of acrylic non-toxic paint (Super Tempera, Fine Art Supplies, New Zealand). The water maze was divided into 8 virtual segments using eight arbitrarily defined compass points marked on the rim of the pool (ie. N, NE, E, SE, S, SW, W, NW). A 10cm circular white perspex escape platform with a non-slip surface was placed in the pool at various positions and distances from the pool edge and sat 3cm below the surface of the water. During the testing phase the room's salient visual cues were visible, such as geometric shapes and high contrast visual stimuli placed on the walls, e.g. small road cones, sink unit, a computer, tables and posters. The testing room also contained a beige curtain hanging from the ceiling on a circular track around the pool that could be opened or closed. A camera fixed to the ceiling above the centre of the pool was used to track swim pathways (Ethovision XT 5.0.212, Noldus Information Technology, The Netherlands). Measures recorded were path-length, escape latency and swim speed. Three CPUs were placed around the room and left running; one of which was the data recording computer. Lighting was provided two overhead fluorescent lights and by a large upward facing lamp (300 watts) on a stand approximately 180cms tall and positioned 40cms from E sector. One additional lamp (60w) was located in the corner of

the room opposite the SW quadrant and was used to keep the rats warm during testing and provided an additional light source.



Figure 6.2: Photograph depicting the water maze used for this experiment.

#### 6.2.9. Reference memory testing

Prior to surgery all rats were trained to locate a platform in the water maze for 4 days. For the 12 days of reference memory testing the rats were trained to swim to a hidden escape platform in a fixed location. For this task the curtain around the pool was open and all extra maze cues were visible. Rats were randomly allocated to one of four fixed platform location 45 cms from the edge of the pool, in either the SW, NW, NE or SE quadrant. Each rat received four trials per day from four stable, but arbitrarily defined compass points N, S, E, W in a randomised sequence. This sequence was varied between days, and between rats to reduce systematic error. For each trial the rat was gently lowered into the water facing the edge of the pool and then released, at which point the tracking software was initiated. A trial finished once the rat had located the platform or 60 seconds had elapsed at which time the rat

was guided to the platform by the experimenters hand and allowed to stay there for a further 15 seconds to orient themselves to the environment. Rats were tested in squad of 3-4 each rat completing a trial before moving on to the next trial giving an inter-trial interval of 3-4 minutes.

#### 6.2.10. Working memory testing in the water maze

For this task a total of 12 platform positions varying in distance from the edge of the pool (35 & 55cms) and 8 release points were used Vann and Aggleton (2003). For this task the platform position remained constant within a session, but varied between sessions so the rats experienced a novel platform location each day. Each rat received four trials per day. The same release point was used for trials one and two for direct comparison, but was varied for trials three and four to examine the flexibility of the memory for the platform location. For this task the rats were randomly divided into two groups. Each group experienced the same release points and platform locations but in the reverse order. Each rat received massed trials in which a trial was terminated after it had reached the platform or 120 seconds (reflecting the increased difficulty of the task) had elapsed at which time it was guided to the platform by the experimenter where it remained for 30 seconds, before being dried with a towel and promptly returned to the pool for its next trial giving an ITI of ~15 seconds. Each rat within a group experienced the same four starting points each day.

Next a procedural modification was used to examine the ability of the rats to use allocentric cues for navigation. For this task the curtains were drawn closed around the pool to conceal the spatial configuration of the room and the allocentric cues fixed to the walls. To obscure features of the ceiling the rats might use for spatial navigation e.g. lights, an air vent and a pulley system for a radial arm maze, material was fixed on top of the curtain rail for form a roof over the pool, leaving only a small square hole for the tracking camera. One salient cue consisting of four distinct objects suspended from a rectangular wooden frame was hung from the curtain rail in line with the N release point. Rats were trained for 6 days in the same fashion as the standard working memory task.

#### 6.2.11. Post surgery radial arm maze testing

#### 6.2.12. Sessions 1-12 standard working memory testing

Rats were given a one day break before being re-habituated to the radial arm maze for 3 days first in cage lots and then singly per rat. The following day testing on the standard working

memory task began. Each rat was tested for 12 consecutive days in the same manner as pre surgery testing.

### 6.2.13. Sessions 13-20 maze rotation and delay tasks.

Following the standard task the rats completed eight days of mid trial delay and delay plus rotation task (four days of each counterbalanced). The initial four arm choice in both the delay and rotation task were identical to the standard working memory task. After the rat had made four correct choices it was gently removed from the maze and placed in a separate cage. Care was taken to ensure the rat was not rotated in anyway. In the delay condition the rat was held in the separate cage until 60 seconds had elapsed at which point it was returned to the central hub in the same spatial orientation it had assumed prior to being removed. The rat was then allowed to carry on making arm choices as per the standard task, until all 8 arms had been visited, 20 arm choices had been made (including those prior to the delay) or 10 minutes had elapsed. The rotation condition was identical except when the rat was removed to the separate cage, the doors of the maze were disconnected from the overhead rigging and the entire maze was rotated clockwise by 45° so that each arm changed position by one place e.g. arm 4 was now where arm 5 was prior to rotation. The food pellets in the four remaining arms were moved to retain their relative position in the environment i.e. the food reward is in a different arm, but the same physical location it occupied prior to rotation. This manipulation is suggested to put the internal and environmental cues in conflict (Vann & Aggleton, 2002; Vann et al., 2004; Pothuizen et al., 2008)

### 6.2.14. Sessions 21-23 immediate early gene activation.

Following a two day break rats were returned to the radial arm maze and were tested on an altered version of the delay plus maze rotation task, which is especially sensitive to retrosplenial cortex function, to drive *zif268* expression in the hippocampal diencephalic circuit (Vann & Aggleton, 2002; Vann, Wilton, Muir, Aggleton, 2003; Pothuizen, Aggleton & Vann, 2008). For this task each rat was allowed to complete 3 massed trials, one trial being 8 arm choices (regardless of outcome). The spatial configuration of the room was changed by altering the position of a curtain on a circular rail hanging around the radial arm maze. New extra maze cues were added and the position of old cues was changed. One configuration was used for the first two sessions to habituate the rats' to changes in the environment and a different novel configuration was used in the third session to drive activation of the extended hippocampal circuit. The first four trials of each session were run in the same manner as the



standard working memory task. After four arm choices (regardless of repeats) the rat was carefully removed from the maze and placed in a separate cage while the entire maze was rotated clockwise 45 degrees, caution was taken to not rotate the rat in any way. The remaining food rewards were moved so that the four unvisited arms retained their position relative to the environmental cues. The rat was returned to the maze after 60 seconds and only allowed four more choices, regardless if they were correct or incorrect. The rat was then removed from the maze and placed in a separate cage for 2 minutes while the maze was reset. The separate cage contained a wooden food receptacle (4 by 5 by 1cms, with a 1cm diameter food well in the centre) in which chocolate pellets were placed during the 2 minute time out. All rats received a minimum of 2 chocolate pellets and received an additional 2 chocolate pellets if they got one or 2 arms wrong or an additional 4 pellets and if they got 3 or 4 arms wrong to equate rewards received between rats. After two minutes the rat was returned to the maze and completed the same procedure another two times. Once the rat had consumed the food rewards after the final session for the day it was moved to a quiet dark room for 90 minutes to facilitate immediate early gene activation.

### 6.2.15. Histological and immunohistochemical procedures

#### 6.2.16. Perfusion

90 minutes after completing the final radial arm maze session (approximately 6 months after lesion surgery) rats were deeply anaesthetised with sodium pentobarbital (125mg/kg) and perfused transcardially, first with ~200mls of chilled saline, followed by ~200mls of 4% paraformaldehyde in a 0.1 M phosphate buffer solution (pH 7.4). The perfused brain was removed and transferred into additional 4% paraformaldehyde solution to post fix overnight. After post fixing, brains were transferred into a long term solution (20% glycerol, 0.1mol PB and 0.05% sodium azide) for a minimum of 48 hours.

Sections were taken in the coronal plane (25µm) from approximately +1.3 to -7.30 from bregma using a sliding microtome with a freezing stage (Thermofisher, UK). The coronal sections were collected in two separate series of six 2ml cryovials. The first series captured the regions of interest for immunohistochemical evaluation. This series included sections from septal region to the anterior thalamus (approximately +1.6 to +1.60 from bregma) and sections posterior to the mammillary bodies to the pre-subiculum (approximately -5.60 to -7.80 from bregma). The second series captured the necessary regions for lesion verification in both MTT and ATN rats. This series included sections



immediately anterior to the ATN through to the posterior mammillary bodies (approximately -0.80 to -5.30 from bregma). In addition, one in every four sections from series two were included in series one to get an even distribution of samples throughout the brain. Tissue sections were stored at -20° c in a cryo-protectant solution (30% glycerol, 30% ethylene glycol and 40% 0.1M PB) until processed for immunohistochemistry or lesion verification.

For ATN lesions, sections were mounted on to gelatinized slides and stained with cresyl violet, a nissl stain, for histological assessment. For MTT lesions, sections were mounted on to gelatinized slides and incubated overnight at 55°c in a 95% ethanol solution containing 0.1% solvent blue, also known as Luxol blue (Sigma, Castle hill, NSW), a myelin specific stain. The slides were then counterstained with cresyl violet, for histological assessment.

#### 6.2.17. ATN lesion verification

Lesion extent was replicated on electronic copies of the Paxinos and Watson (1998) atlas (see Mitchell and Dalrymple-Alford, 2005). Automated pixel counts of the estimated damage relative to the relevant intact brain region were used to generate percent lesion volumes by factoring in the pixel areas multiplied by the distances provided in the atlas. Acceptable lesions were defined as having more than 50% bilateral damage to the ATN, minimal fornix damage, and not more than 40% damage to the corresponding adjacent lateral thalamic region (LT, which included the intralaminar and lateral mediodorsal nuclei) and posteromedial thalamic region (MT, which included the medial and central mediodorsal nuclei). The latter regions were of interest because they have also been suggested as possible causes of some aspects of diencephalic amnesia, but only ATN lesions of more than 50% damage size are consistently associated with severe spatial memory deficits (Gibb et al., 2006; Mitchell & Dalrymple-Alford, 2005; 2006; Bailey & Mair, 2005).

#### 6.2.18. MTT lesion verification

MTT lesions were assessed using luxol blue (a myelin specific stain) and cresyl violet stained sections, any cases with unilateral or bilateral sparing were removed from further analysis.

### 6.2.19. Zif268 DAB immunostaining

Endogenous peroxidase activity was blocked by washing the tissue sections with 3% hydrogen peroxide in 0.1M phosphate buffered saline containing 0.3% Triton X-100 (PBST) for 10 minutes. The tissue sections were then washed four times in PBST alone for 5 minutes each before non-specific binding was blocked by incubating the tissue sections for 60 mins with 10% normal goat serum (NGS; Life technologies, NZ). Excess blocking solution was removed with  $4 \times 5$  min washes in PBST before sections were incubated for 48 hours at 4 °C with a rabbit polyclonal zif268 antibody (also known as Egr-1; 1:3000; Santa Cruz Bio) in PBST with 1:100 NGS added. Tissue was then rinsed in PBST for 5 minutes, four times. Next, they were incubated in a biotinylated goat anti-rabbit secondary antibody (1:400; Vector) with 1:100 NGS, and then avidin-biotin horseradish peroxidase complex in PBST (Vector elite kit). Sections were then rinsed for 10 mins in 0.05mol Tris buffer (pH 7.4), four times. Finally, immunoreactivity was visualised with diaminobenzidine (Sigma, Castle Hill, NSW) in tris buffer with 0.00013% hydrogen peroxide added just prior to incubation. Sections were then mounted on gelatinized slides allowed to dry and then run through graded alcohols before being cleared in xylene and coverslipped with DPX.

### 6.2.20. NeuN immunofluorescence

Non-specific binding was blocked with 10% normal horse serum (NHS) in PBST for one hour. Sections were then rinsed for 5 mins in PBST, four times, and then incubated for 24 hours at 4 °C with a monoclonal mouse anti-NeuN antibody (1:1000, Millipore) in PBST with 1:100 NHS added. Following incubation, sections were washed for 5 mins in PBST, four times and then incubated in a fluorescent secondary (anti-mouse dylight 549 vector at 1:1000) in PBST with 1:100 NHS added, for four hours in the dark. Finally, sections were rinsed for 5 mins, four times, before being mounted to gelatinized slides. The slides were then cover slipped with Fluoromount (sigma, Castle Hill) and sealed with clear nail varnish. The slides were left overnight to dry and were then stored at 4°C in the dark until NeuN expression was visualised.

### 6.2.21. Zif268 Quantification

For zif268 immunostaining sections were viewed on a Nikon E800 microscope 4X objective, and photographed using a Nikon DS-Fil camera. Automated counts of the stained cells were obtained using the public domain NIH image program (developed at the US National

Insitutes of Health and available on the internet at <http://rsb.info.nih.gov/nih-image/>). Cell counts were taken without knowledge of group assignments. Images were gray-scaled, and the cell detection threshold was set automatically with the build in thresholding algorithms. With few exceptions (e.g. a particularly lightly stained section), the threshold algorithm was the same for all sections from the same processing batch. Counts of labelled nuclei in each region of interest were determined by counting those immunopositive cells that were above the detection threshold and between 5 – 20  $\mu\text{m}$  in size. For all brain regions analysed between two and four sections per hemisphere were captured. These counts were combined to give a mean result and all counts were expressed as cells per  $\text{mm}^2$  to control for differences in the size of the region analysed across tissue sections.

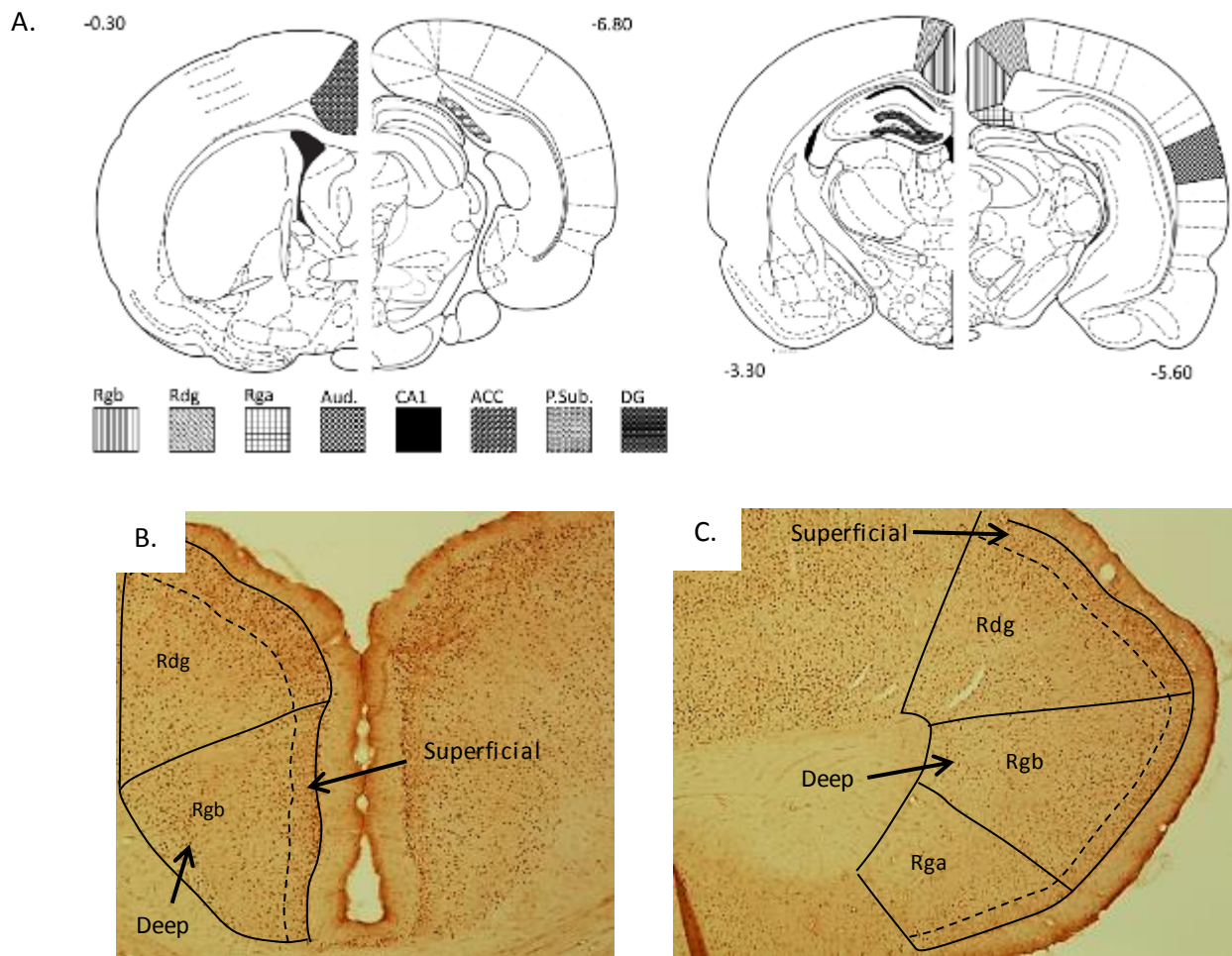


Figure 6.3: Regions of interest for Zif268 immunostaining. (A) Atlas plates from Paxinos & Watson, (1998) indicating the relative location (from bregma) of the cortical and hippocampal regions of interest. (B) Photomicrograph of a Zif268 stained section indicating the subregions of rostral retrosplenial cortex in a sham rat. (C) Photomicrograph of a Zif268 stained section indicating the subregions of caudal retrosplenial cortex in a sham rat. Rgb = granular b retrosplenial cortex, Rdg = dysgranular retrosplenial cortex, Rga = granular a retrosplenial cortex, Aud. = auditory cortex, CA1 = CA1 of the hippocampus, ACC = anterior cingulate cortex, P.Sub = post-subiculum, DG = dentate gyrus of the hippocampus.

### 6.2.22. NeuN quantification

To examine whether MTT or ATN lesions reduced the number of mature neurons in the mammillary bodies, images capturing NeuN expression in the entire mammillary body region were taken at two different anterior-posterior coordinates, approximately -4.52 and -4.8 from bregma (See figure 6.4). At least two approximately adjacent sections (per A.P. coordinate) were examined for each rat. NeuN expressing cells were excited with 549nm light from an Olympus BX51 epi-fluorescence microscope. Cell counts were taken without knowledge of group assignments. Images were gray-scaled, and the cell detection threshold was set automatically. With few exceptions (e.g. a particularly lightly stained section), the threshold was the same for all sections from the same processing batch. Counts of labelled nuclei in each of the lateral mammillary nucleus (LatMB), the medial aspect of the medial mammillary nucleus (MMB\_M) and the lateral aspect of the medial mammillary nucleus (MMB\_L) of interest were determined separately by counting those immunopositive cells that were above the detection threshold and between 5 – 20  $\mu\text{m}$  in size. Total NeuN positive cell counts were averaged across hemispheres and the two AP coordinates to yield a single value for each of the LatMB, MMB and MLMB in each rat.

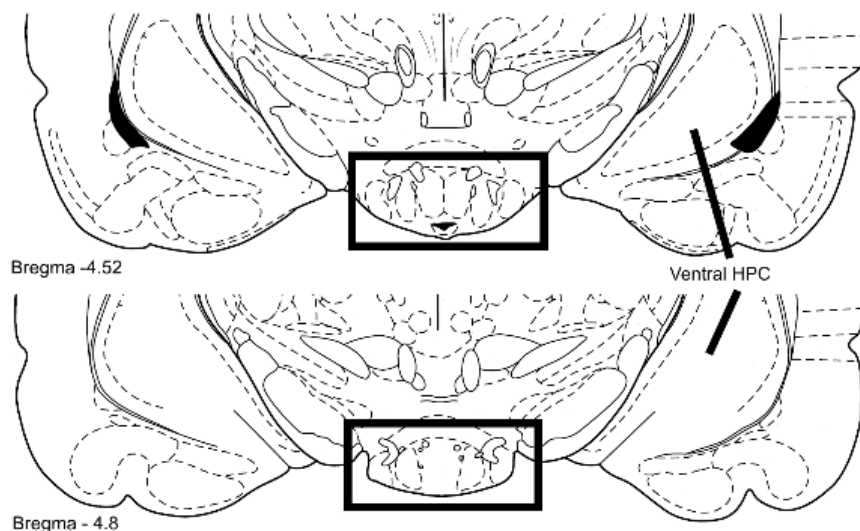


Figure 6.4: Atlas plates from Paxinos & Watson indicating the two locations that NeuN expression was quantified in the mammillary bodies (MB; black boxes).

### 6.2.23. Data analysis

Repeated measures and oneway ANOVAs were employed for all behavioural data and comparisons of Zif268 immunostaining in cortex and hippocampus and NeuN immunostaining in the MB. Significant main effects and interactions were further examined with Newman-Keuls post hoc tests. The critical alpha value was set at 0.05 for all statistical tests.

## 6.3. Results

### 6.3.1. Lesion verification

Nine of the 16 rats given MTT lesions met the criterion of at least 50% bilateral transection of the tract. Most of these successful lesions were complete or almost complete lesions of the tract (range = 89 – 100 %, Fig 6.5). The excluded MTT lesion rats comprised two rats that had complete unilateral MTT damage but insufficient damage to the contralateral MTT (100% on one side, but 45% and 30% on the contralateral side respectively), two rats with partial bilateral damage (30%, 66.6% total damage respectively) due to the focus of the lesion being too ventral or lateral, and two rats with complete bilateral sparing of the MTT. None of the MTT cases had evidence of any damage to the post-commissural fornix, the supramammillary nuclei, MB, or mammillo-tegmental tract. For the 13 rats in the ATN lesion group, ten met the criterion of >50% bilateral ATN lesions and minimal damage to the fornix (Table X); The smallest (67.8%) and largest (99.1%) successful lesions are shown in Fig X. One excluded ATN rat had substantial Fx damage and two ATN exclusions had lesions below the 50% criterion. No differences on any behavioural task were found between the MTT Sham lesion group (n = 14) and the ATN Sham lesion group (n = 11) so these rats were combined for all analyses (Sham, n = 25).



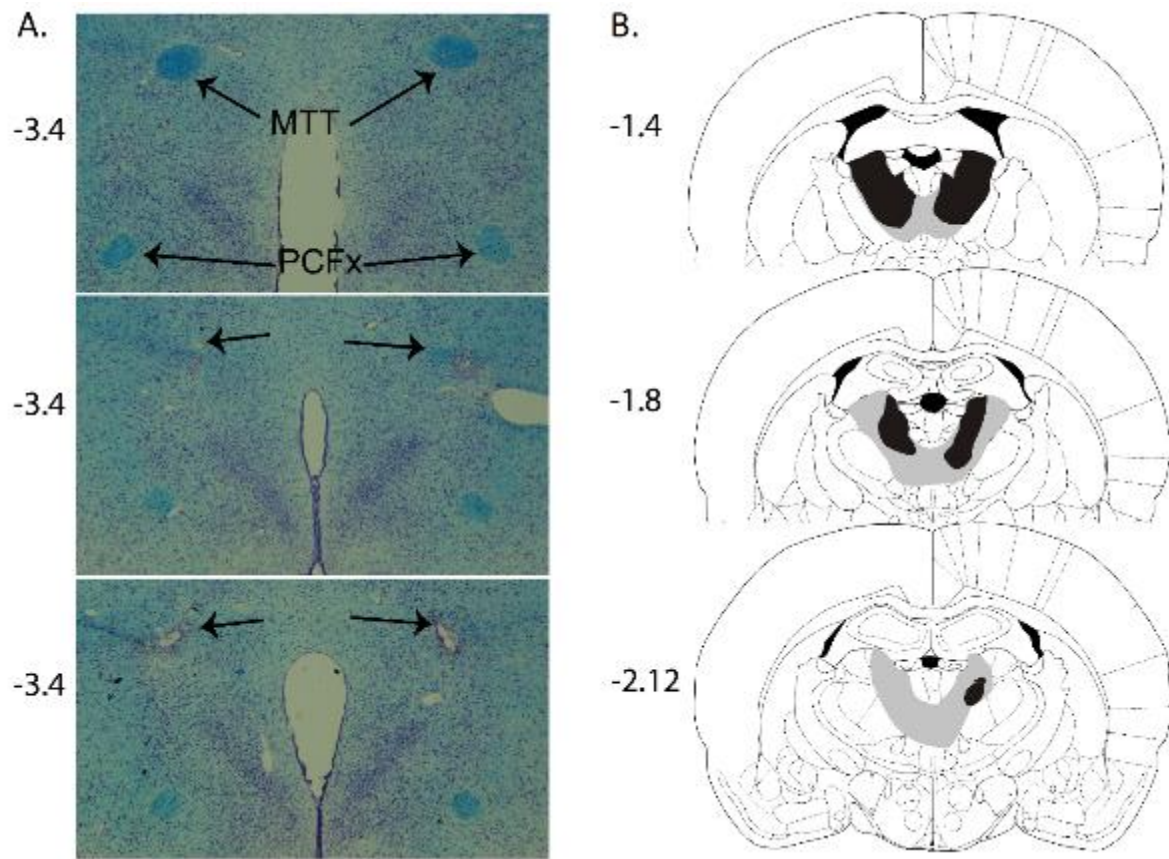


Figure 6.5: (A) Photomicrographs of luxol blue (myelin specific) and cresyl violet (Nissl stain) stained sections showing an intact MTT (Sham; top image), the smallest included MTT lesion (middle image) and a total MTT lesion (bottom image). (B) Atlas plates through the ATN showing the largest (grey) and smallest (black) acceptable ATN lesions.

**Table 6.1: Lesion damage analysis for ATN rats (all values in percentage total volume)**

	ATN					Other ROI's		Other Nuclei				
	Rat	ATN	AD	AM	AV	MT	LT	IAM	LD	PT	Re	Rh
<b>Included</b>	R3	76.8	80.6	64.8	76.6	0.2	8.7	32.3	1.8	3.5	1.4	0.6
	O4	94.1	99.7	79.9	94.8	6.2	37.5	75.0	21.8	4.1	3.2	8.5
	A1	67.8	86.2	46.6	68.7	0.0	6.6	3.8	0.1	4.3	0.4	0.1
	M3	76.6	67.8	58.3	75.5	24.0	48.4	51.2	2.2	3.5	0.1	5.6
	F4	81.4	89.6	98.0	63.0	3.2	39.3	88.1	3.7	8.1	13.9	35.9
	V3	99.1	99.7	99.9	98.9	5.6	47.4	100.0	10.1	11.4	26.1	79.5
	P3	84.8	89.3	80.3	83.8	0.0	10.8	36.4	6.5	6.7	3.3	6.2
	K3	81.2	91.9	72.8	80.7	0.0	9.1	43.7	5.7	5.9	2.2	5.6
	K1	98.5	99.9	99.5	96.8	6.3	46.4	100.0	21.3	6.2	19.3	60.7
	V1	93.0	100.0	85.9	94.2	12.0	52.0	69.9	15.0	3.0	5.7	8.8
<b>Mean</b>		85.3	90.5	78.6	83.3	5.8	30.6	60.0	8.8	5.7	7.6	21.2
<b>STD</b>		10.5	10.5	18.2	12.5	7.5	19.2	31.9	8.0	2.6	9.0	28.0
<b>Excluded</b>												
	R2	38.0	63.8	29.3	36.9	0.0	2.9	0.0	0.0	2.0	0.0	0.0
	V2	38.5	29.4	43.8	36.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0

**Abbreviations:** AD= anterodorsal nucleus; AM= anteromedial nucleus; ATN = anterior thalamic aggregate comprising the anterodorsal, anteromedial and anteroventral thalamic nuclei; AV= anteroventral nucleus; IAM= interanterodorsal nucleus; LT= lateral medial thalamic aggregate comprising the intralaminar nuclei (centrolateral, paracentral and rostral central medial nuclei) and lateral mediodorsal thalamic nuclei (lateral and paralamellar nuclei); LT median= median percent damage for all included rats; MT= posteromedial thalamic aggregate comprising the central and medial mediodorsal nuclei and the intermediodorsal nucleus; MT median= median percent damage for all included rats; PT= paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus; ROI's= regions of interest.



### 6.3.2. Spatial working memory in the 8-arm radial arm maze (RAM)

The future lesion groups showed similar pre-surgery acquisition of the standard RAM task. By the last two-trial block of pre-surgery testing, all groups showed accurate performance with an average of less than one error in the standard RAM task (ATN, = 0.35 (0.52) ; MTT, = 0.58 (0.82); Sham, = 0.61 (0.58)). There was no pre-surgery Lesion effect ( $F < 1$ ), a clear effect across the 6 pre-surgery blocks ( $F(5,205) = 23.41, p < 0.001$ ) reflecting task acquisition, and no Lesion by Block interaction ( $F < 1$ ). By contrast, post-surgery testing in the standard RAM task revealed a clear Lesion effect ( $F(2,41) = 57.93, p < 0.001$ ; Fig 6.6), with the ATN group showing far higher errors than both the MTT group ( $p < 0.001$ ) and the Sham group ( $p < 0.001$ ). The MTT group showed a relatively milder but consistent impairment compared to the Sham group ( $p < 0.001$ ). Both the MTT group and, especially, the Sham group showed improved performance across post-surgery testing, whereas the ATN group maintained poor performance across trial blocks (Lesion x Block,  $F(10, 205) = 2.96, p < 0.01$ ).

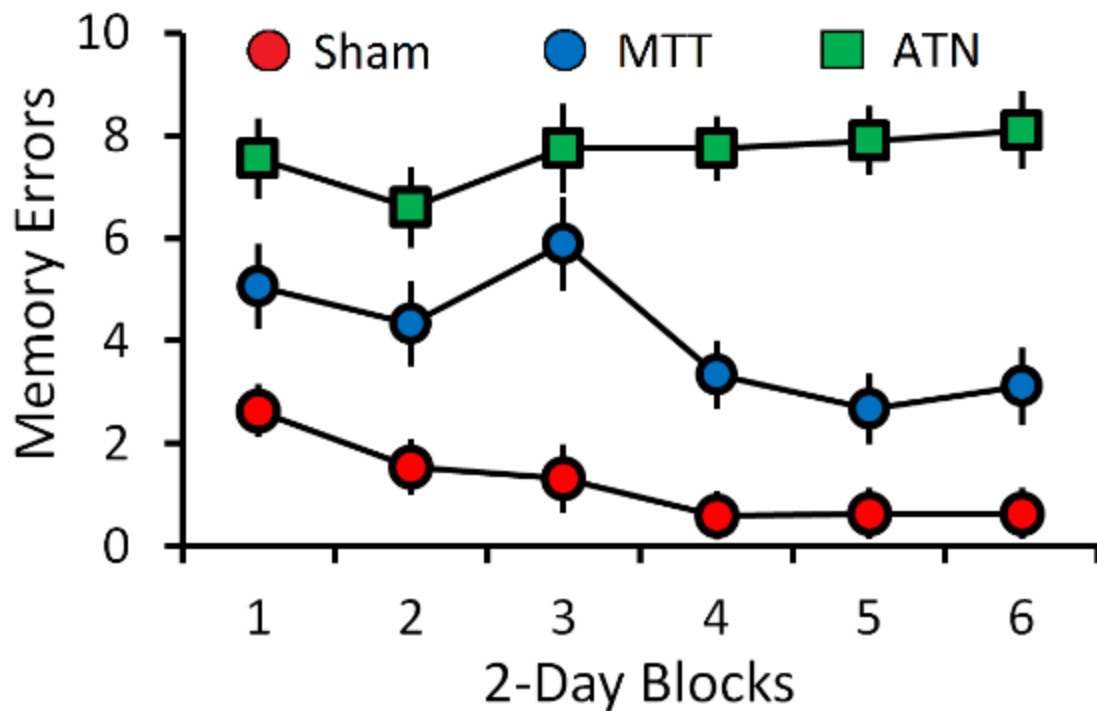


Figure 6.6: Mean  $\pm$ SEM spatial working memory errors for the 12 days of post-surgery testing on the standard RAM.

#### 6.3.4. Mid-trial delay and mid-trial delay + rotation in the RAM

When rats were tested using the 60s delay after the first four choices and the delay plus rotation condition after the first four choices, worse performance was displayed by the MTT group compared to their performance on the previous 4 standard (no-delay) trials, whereas the ATN group appeared to show some benefit of having only a mid-trial delay (Fig 6.7). The Sham group was unaffected by these new conditions. These observations were supported by significant Lesion ( $F(2,41) = 106.65, p < 0.001$ ) and Lesion by Condition ( $F(4,123) = 7.01, p < 0.001$ ) effects. The MTT group showed significantly increased errors for the delay-only trials ( $p < 0.005$ ) and delay plus rotation trials ( $p < 0.001$ ) compared to their standard trials, but not between the two non-standard trials ( $p < 0.10$ ). The ATN group showed significantly decreased errors for the delay-only condition compared to their standard trials ( $p < 0.001$ ), but performance across the standard trials and the delay plus rotation trials did not differ in the ATN group ( $p > 0.2$ ).

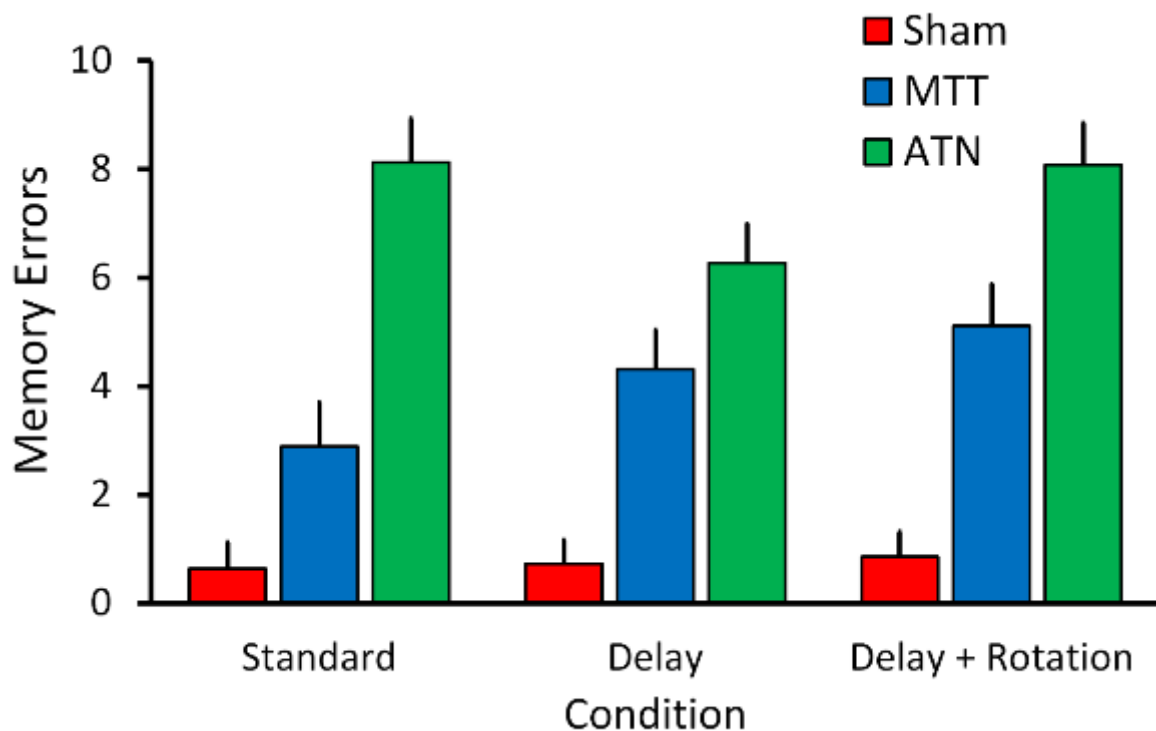


Figure 6.7: Mean + SEM spatial working memory errors for the last 4 days of the standard post-lesion RAM task, the 4 days of mid-trial delay testing and the 4 days of mid-trial delay with rotation testing.

### 6.3.5. Spatial reference memory in the water-maze

Rats were first tested in the classic reference memory task (Fig 6.8). The ATN group took a longer path length to find the submerged platform than both the MTT group and the Sham group (Lesion,  $F(2,41) = 22.45, p < 0.001$ ). The pairwise difference between the ATN and the other two groups was significant ( $p < 0.001$ ), but the overall mean difference between the MTT group and the Sham group did not reach significance ( $p < 0.10$ ). Across trial blocks, there was an overall reduction in path length taken (Block main effect,  $F(5,205) = 51.11, p < 0.001$ ), but the Lesion by Block interaction was not significant ( $p > 0.4$ ).

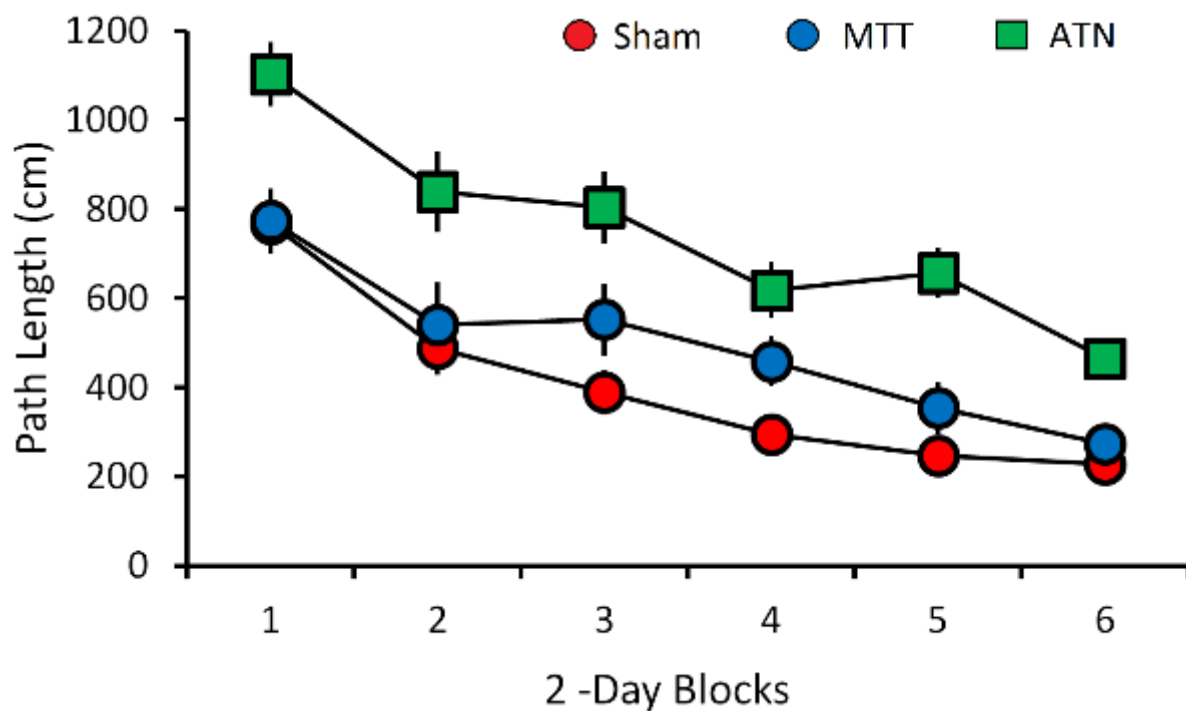


Figure 6.8: Mean  $\pm$  SEM path length to the hidden escape platform across the 12 days of reference memory testing in the water maze.

### 6.3.6 Spatial working memory in the water-maze

When tested in the working memory water-maze task, in the same room conditions as used for reference memory testing, a Lesion effect was again evident ( $F(2,41) = 25.32, p < 0.001$ ; figure 6.9). Unlike reference memory, however, both MTT and ATN groups showed significantly longer path lengths than the Sham group in the working memory task ( $p < 0.001$  and  $p < 0.001$ ), and the two lesion groups did not differ ( $p > 0.1$ ). The typical working memory performance of reduced path length across trials within session was clear in the Sham group, but less evident in both lesion groups (Lesion  $\times$  Trial,  $F(6, 123) = 4.02, p < 0.002$ ).

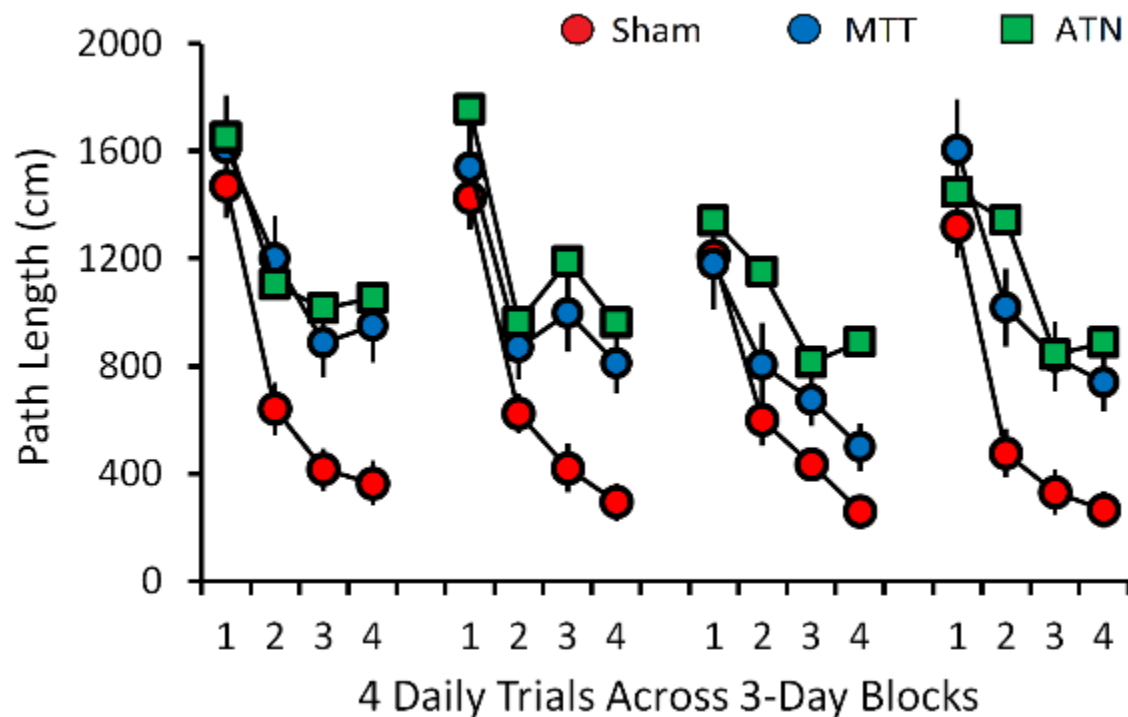


Figure 6.9: Mean  $\pm$  SEM path length to the hidden escape platform across the four daily trials in the working memory water maze task.

### 6.3.7. Spatial working memory in the water-maze with reduced spatial cues

When subsequently tested in the working memory water-maze task, but now with the visual room cues minimised, the Lesion main effect was replicated ( $F(2,41) = 45.47, p < 0.001$ , Figure 6.10), with both lesion groups again impaired relative to the Sham group ( $p < 0.001$ ,  $p < 0.001$ ), but the ATN group was now significantly worse than the MTT group ( $p < 0.001$ ). Once more, the impairment in the lesion groups was primarily due to their poorer working memory across trials within session (Lesion  $\times$  Trial,  $F(6,123) = 4.11, p < 0.001$ ). The increased impairment in the ATN group relative to the MTT group was due primarily to differences in the second block of trials (Lesion  $\times$  Block,  $F(2,41) = 4.18, p < 0.05$ ; Lesion  $\times$  Block  $\times$  Trials,  $F(6,123) = 3.23, p < 0.005$ ), with the MTT group showing shorter path lengths on the third and fourth trials for the second half of this test.

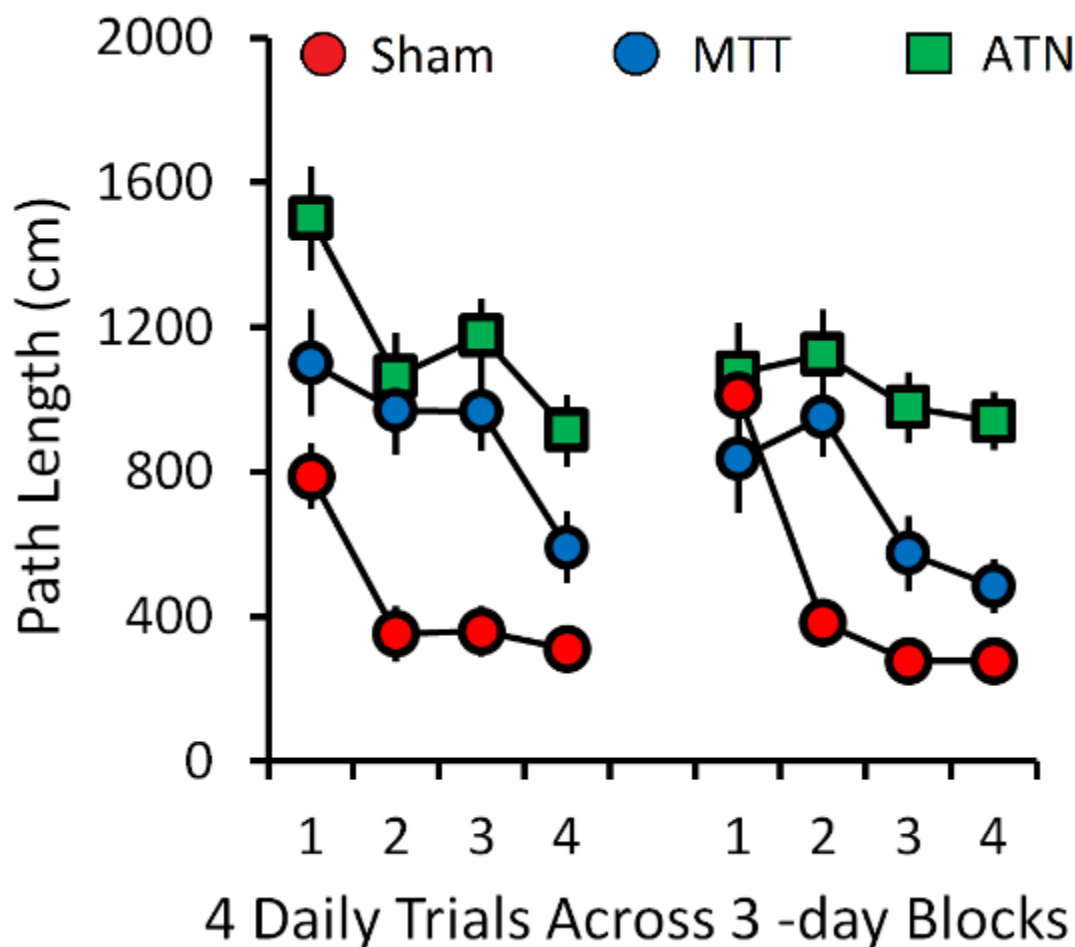


Figure 6.10: Mean  $\pm$  SEM path length to the hidden escape platform across the 4 daily trials in the working memory water-maze task with reduced cues.

### 6.3.8. Massed “delay plus rotation” in the RAM to drive zif268 expression

Zif expression was stimulated by having the rats conduct a modified delay plus rotation condition on the day of sacrifice, in which novel room cues were used and 3 consecutive trials were conducted but the rats were given a maximum of 4 arm visits after the delay period on each trial. They also had two similar days of yet another set of novel room cues (same on these two first days) prior to the last zif-induction day. Changing the room cues and minimising arm visits led to a reduction in the relative deficit shown by rats with lesions (Figure 6.11). Overall, rats tended to make more errors on the second and third trial, perhaps due to increased proactive interference (Trial main effect,  $F(2,82) = 4.06, p < 0.05$ ). Lesion groups made more errors than Shams (Lesion,  $F(2,41) = 20.07, p < 0.001$ ) with the ATN group making significantly more errors than the MTT group ( $p < 0.05$ ).

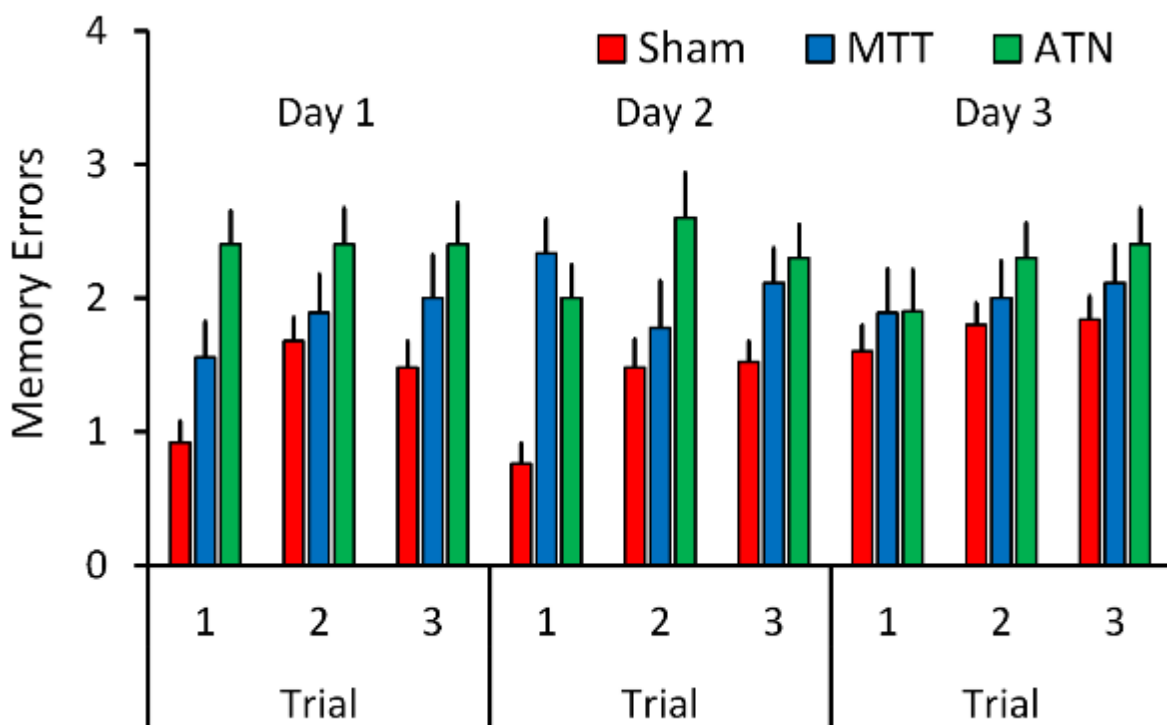
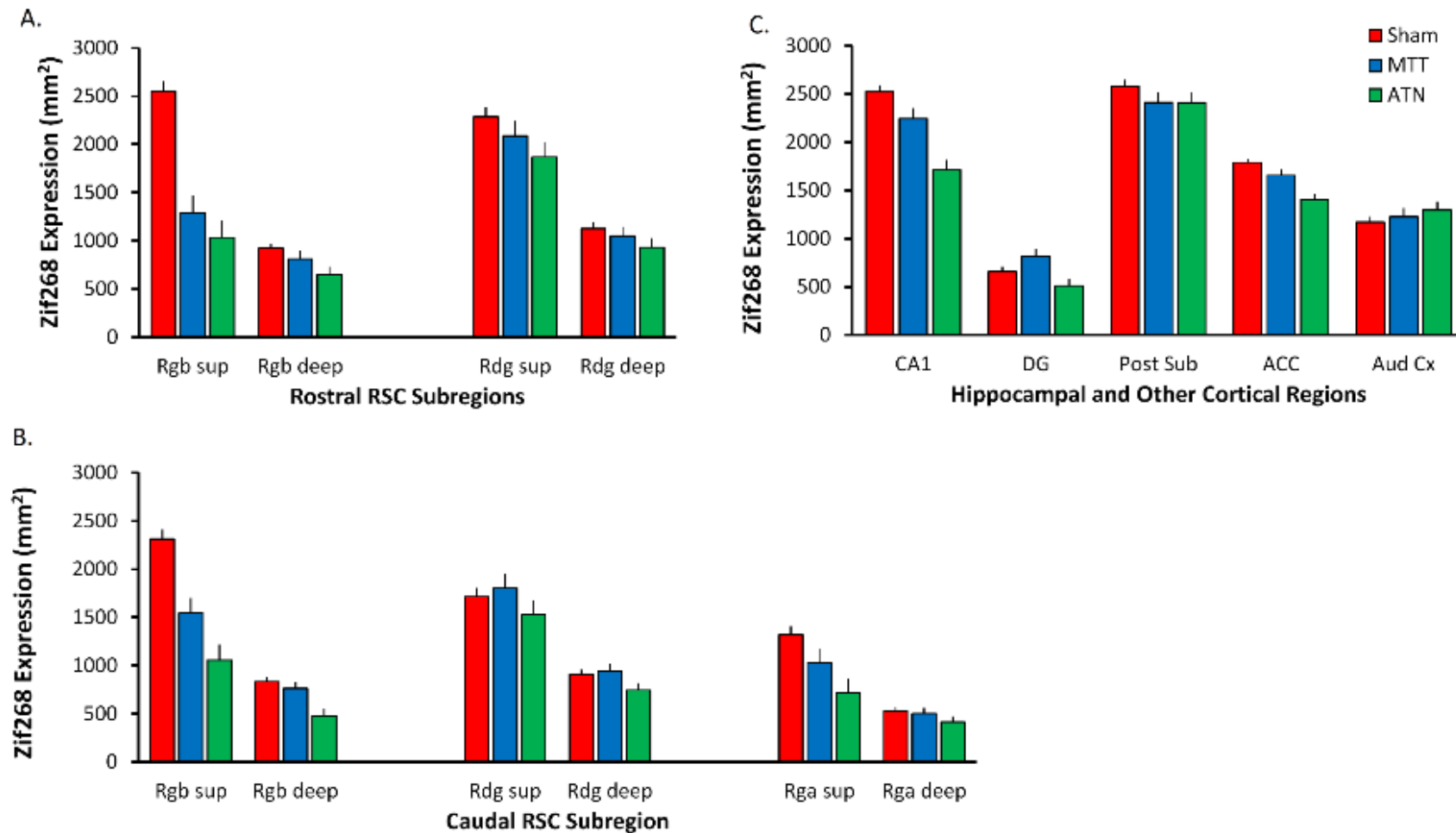


Figure 6.11: Mean + SEM spatial working memory errors in the final mid trial delay + rotation RAM task used to drive Zif268 expression in the extended hippocampal circuit.

### 6.3.9. Zif268 in RSC and hippocampus

Each region of interest was analysed using separate between groups ANOVA of zif268 counts per mm-sq, to minimise the confound of interpreting complex interactions across multiple sites. Of primary interest was expression in the retrosplenial cortex (RSC). Both MTT and ATN lesions resulted in substantially reduced zif268 expression in both the rostral ( $F(2,41) = 53.81, p < 0.001$ , figure 6.13) and caudal ( $F(2,41) = 33.22, p < 0.001$ ) superficial layer of the Rgb (figure 6.12 A & B). This change was equivalent across the two lesion groups in the rostral superficial Rgb, but significantly worse in the ATN group in the caudal superficial Rgb,  $p < 0.01$ ). Only ATN lesions significantly reduced zif268 expression in the deep layers of the Rgb (rostral,  $F(2,41) = 7.14, p < 0.005$ ; caudal,  $F(2,41) = 14.54, p < 0.001$ ; ATN vs sham,  $p < 0.004$ ; MTT vs sham,  $p > 0.1$ ). The only other significant Lesion effects were in the superficial layer of the rostral Rdg ( $F(2,41) = 3.84, p < 0.05$ ) and the (caudally located) superficial Rga ( $F(2,41) = 7.85, p < 0.005$ ), which in both cases reflected significant Zif268 reductions in the ATN group only ( $p < 0.05$  ATN versus Sham;  $p > 0.05$ ). ATN lesions also reduced zif268 expression in an additional cortical region, the anterior cingulate ( $F(2,41) = 8.01, p < 0.01$ ), both in relation to the sham group ( $p < 0.005$ ) and MTT group ( $p < 0.05$ ); the MTT group did not differ significantly from Shams ( $p > 0.2$ ). No effect of lesion was found in the cortical control region. Lesions did not affect zif268 expression in the post-subiculum ( $p > 0.1$ ). Lesions resulted in differential effects in the dentate gyrus ( $F(2,41) = 4.8, p < 0.05$ ), with the ATN group showing significantly less expression than the MTT group ( $p < 0.005$ ), but neither lesion group differed from Sham ( $p > 0.06$ ). There was also a significant Lesion effect for the CA1 ( $F(2,41) = 23.66, p < 0.001$ ; figure 6.13) where ATN lesions reduced zif268 in the CA1 relative to the MTT and Sham groups (both  $p < 0.001$ ; more strongly than did MTT lesions, MTT vs Sham,  $p < 0.05$ ).

Figure  
6.12:  
Mean  
+  
SEM  
Zif268



positive cell counts per mm squared in (A) the rostral subregions retrosplenial cortex, (B) the caudal subregions of the caudal retrosplenial cortex and (C) in the hippocampal and other cortical sub regions. **Abbreviations:** Rgb = granular b retrosplenial cortex, Rdg = dysgranular retrosplenial cortex, Rga = granular a retrosplenial cortex, Aud. = auditory cortex, CA1 = CA1 of the hippocampus, ACC= anterior cingulate cortex, P.Sub = post-subiculum, DG = dentate gyrus of the hippocampus.



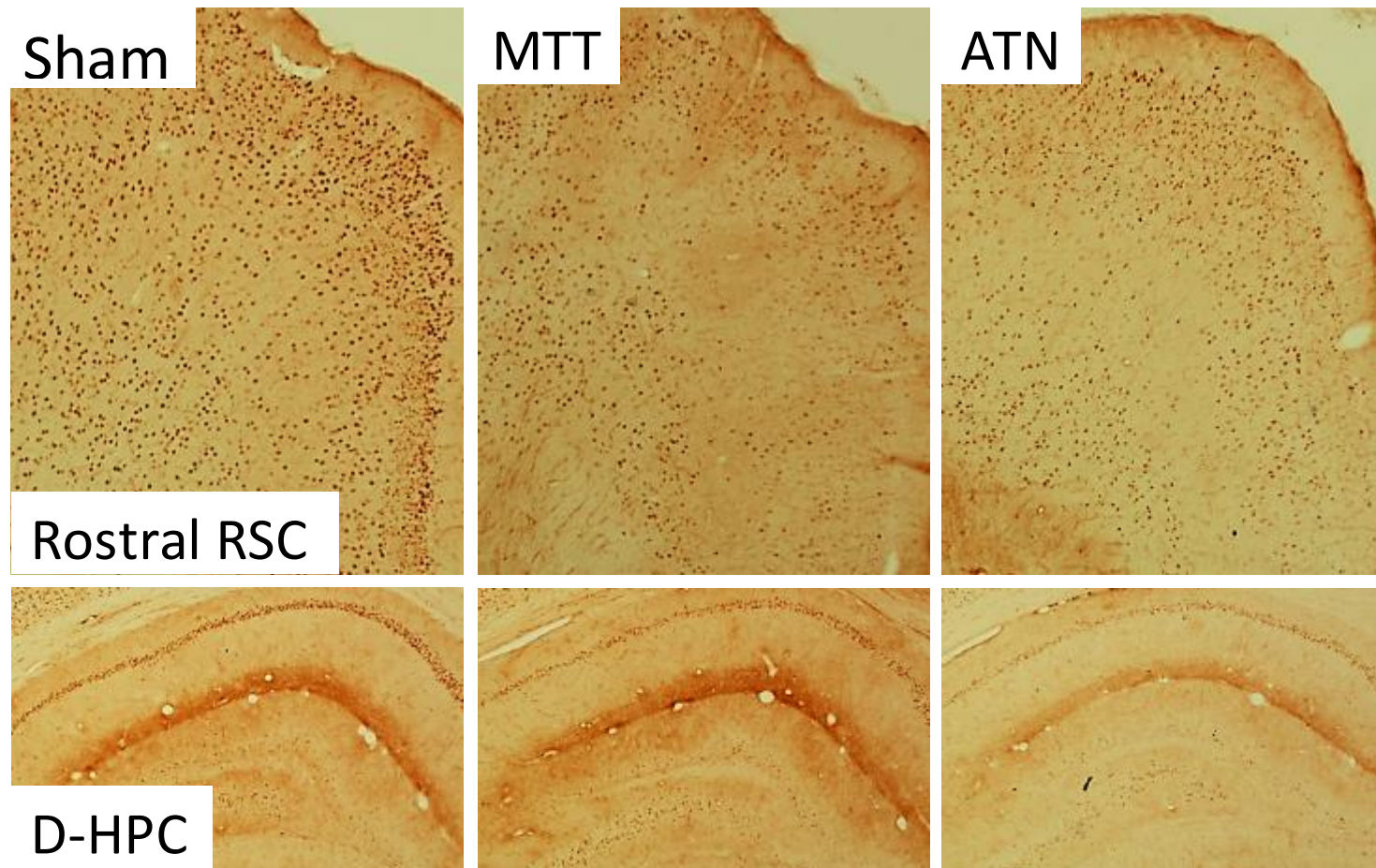


Figure 6.13: Example photomicrographs of zif268 immunostaining in the rostral retrosplenial cortex (top panel) and the dorsal hippocampus (bottom panel) from a sham (left), MTT (middle) and ATN (right) rat.

### 6.3.10. NeuN expression in the MB

As for zif268 expression, each region of interest was analysed using separate between groups ANOVA of NeuN counts in the MB to minimise the confound of interpreting complex interactions across multiple sites. Both MTT and ATN groups had substantially reduced NeuN positive cell counts in the medial part of the medial MB (Lesion,  $F(1,40) = 69.76$ ,  $p < 0.001$ ), the medial lateral part of the media MB (Lesion,  $F(1,40) = 137.90$ ,  $p < 0.001$ ) and the lateral MB (Lesion,  $F(1,40) = 69.48$ ,  $p < 0.001$ ). The MTT group showed a greater reduction than the ATN group in all three subregions ( $p < 0.001$ , figures 6.14 & 6.15).

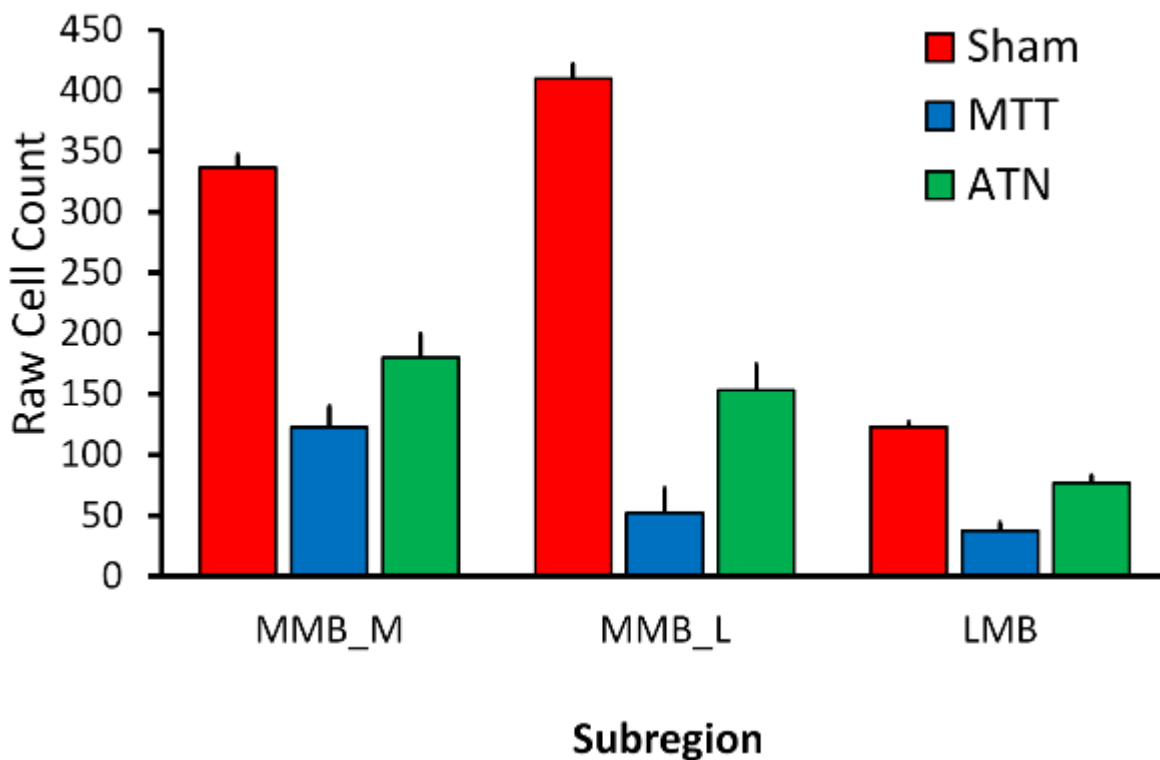


Figure 6.14: Mean +SEM raw NeuN positive cell counts across the two medial and the lateral subcomponents of the mammillary bodies. MMB\_M = medial mammillary bodies pars medialis, MMB\_L = medial mammillary bodies pars lateralis, LMB = lateral mammillary bodies.

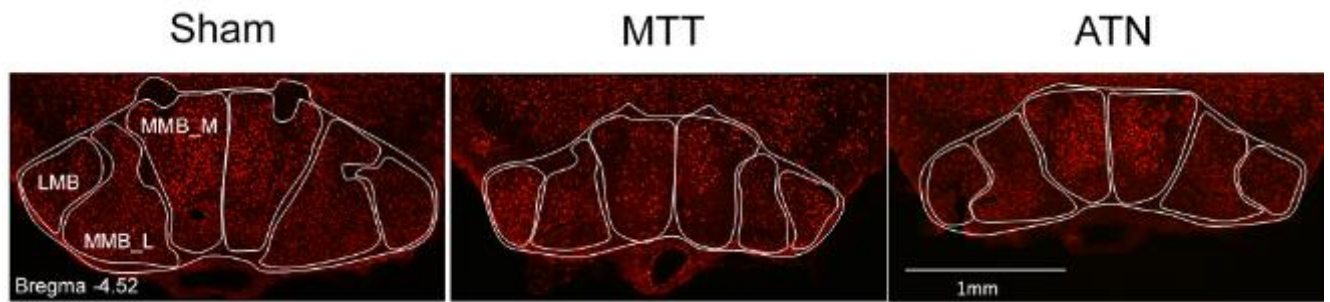


Figure 6.15. Example photomicrographs showing NeuN positive staining in the two medial and the lateral subdivisions of the MB in a sham, MTT and ATN lesion rat.

### 6.3.11. Associations between histology and behaviour

In the ATN group damage to the medial dorsal nuclei (range; 0-24%) and the intralaminar nuclei (range; 6.6-52%) were correlated against total spatial working memory errors in the standard RAM to verify whether non-target damage was related to behavioural performance. Across rats, neither damage to the MT ( $r = 0.2$ ,  $p > 0.1$ ), nor damage to the LT ( $r = 0.003$ ,  $p > 0.9$ ) was related to spatial working memory errors in the RAM.

## 6.4. Discussion

This study is the first to provide a direct comparison of the neural and behavioural effects of bilateral lesions to the MTT and the ATN, two key structures within the neuroanatomy of an extended hippocampal system (Aggleton & Brown, 1999; Aggleton, 2008). Neuropathology in both of these regions has been strongly implicated as causal factors in clinically-defined dense amnesia (Harding et al., 2000; Van der Werf et al., 2000,2003; Carlesimo et al., 2011; Kopelman, 2014; Dzieciol et al., 2017), but their relative influence is uncertain. The strongest clinical evidence to implicate the ATN comes from KS patients, which resonates with the view that the ATN complex represents a pivotal structure within the extended brain network responsible for episodic recollection (Aggleton et al., 2010; Jankowski et al., 2013). KS patients, however, invariably have additional MB and thalamic pathology and often cortical neuropathology (Harding et al., 2000; Savage et al., 2012; Kopelman, 2014). Recent animal work, however, has suggested that there needs to be a greater focus on the influence of brainstem structures on the memory system upstream, via their impact on the ATN, which suggests that damage to the MTT afferents to the ATN would produce comparable memory impairments to that found after ATN lesions (Vann, 2013; Dillingham et al., 2015; Vann & Nelson, 2015). This latter perspective places an emphasis on the association between MB and MTT neuropathology and clinical amnesia.

Our direct comparison, however, showed that ATN lesions clearly resulted in a wider range of spatial memory deficits compared to those of MTT lesions and when a task revealed impairments after both lesions then there was often a more severe deficit after ATN lesions. ATN lesions produced a marked spatial reference memory deficit in the water maze, whereas rats with MTT lesions did not differ from shams on this task. MTT and ATN lesions resulted in an equivalent spatial working memory deficit in the water maze under standard testing conditions, but when the spatial cues in the room were minimised then the ATN group again showed a greater deficit than that shown by the MTT group. Both MTT and ATN lesions impaired spatial working memory performance on the standard 8-arm RAM task, but this deficit was substantially greater in the ATN group. The addition of both a delay after the first four arm entries and a mid-trial delay plus rotation condition increased RAM errors made by rats with MTT lesions, but their impairment remained less severe than that of the rats with ATN lesions. Compared to the standard RAM procedure, rats with ATN lesions were if

anything aided by the addition of a mid-trial delay and not made any worse when the maze was rotated by 45 degrees during the mid-trial delay.

Neurobiological measures also showed differences associated with the two lesions. To measure neuronal activation, *zif268* expression was stimulated using test conditions that are theoretically sensitive to the functions of the extended hippocampal system, especially the RSC (Dillingham et al., 2015; Vann & Nelson, 2015). Both MTT and ATN lesions resulted in a striking loss of *zif268* expression in the superficial layers of the Rgb, which replicates previous findings (Poirier & Aggleton, 2009; Dumont et al., 2012; Frizzarti et al., 2016). Such evidence supports the view that the MB, which do not have direct afferent connections with distal cortico-limbic structures, have a critical influence on these upstream regions and that pathology of the MB and MTT therefore has a profound impact on the integrity of the extended memory system (Dillingham et al., 2015; Vann & Nelson, 2015). However, only ATN lesions reduced expression in the deep Rgb, the superficial Rdg and the superficial Rga subregions of this limbic cortex, which suggests a broader impact of ATN injury that may reflect the direct interconnections that exist between the ATN and the RSC. Some effects on *zif268* were also observed in the anterior cingulate cortex and hippocampus. Again however, only ATN lesions reduced *zif268* expression in the anterior cingulate cortex had a greater impact on hippocampal CA1 than MTT lesions.

The relative importance of MB pathology on memory and cortico-limbic *zif268* expression can be discerned from the impact of MTT and ATN lesions on neuron-positive (NeuN) cell counts in the MB. The influence of ATN lesions on MB cell counts has not been reported previously, although previous work on MTT lesions has suggested cell loss on the basis of reduced MB volume without increased cell density (Vann & Aggleton, 2003; Vann, 2013). Prior evidence that MTT lesions deafferent the anteroventral and anteromedial nuclei but leave relatively intact the anterodorsal nuclei afferents from the MB (Vann & Albasser, 2009) suggested that MTT lesions would have less influence on the MB, and thereby the MB-ATN axis, than would direct ATN lesions that target all three subcomponents of the ATN complex. In fact, we found that ATN lesions did cause cell loss across all three subcomponents of the MB complex, but in this instance it was MTT lesions that caused greater cell loss irrespective of MB subregion. Hence the greater impact of ATN lesions on behaviour and *zif268* in the upstream cortico-limbic system does not co-vary with the relative influence or extent of MB neuropathology. Interestingly, proportional cell loss was greater in

the lateral aspect of the medial MB after both MTT and ATN lesions. The lateral aspect of the medial MB projects primarily to the anteroventral nucleus of the ATN, but the behavioural significance of depleted MB neurons in this region is not known. The greater behavioural deficits after ATN lesions than after MTT lesions was also not a function of the relative integrity of each target structure, because MTT lesion size tended to be more complete than was the case for ATN lesions, which corresponds with the impact of each lesion type on MB integrity.

Previously, only separate studies have been available to estimate the comparative effects of MTT and ATN lesions on memory tasks. The similar effects of both lesion types have been emphasised, in that neither lesion impairs novel object discrimination (Moran & Dalrymple-Alford, 2003; Warburton and Aggleton, 1999; Nelson & Vann, 2014), but both lesions impair temporal memory for a sequence of items (Wolff et al., 2008; Dumont & Aggleton 2013; Nelson & Vann, 2016) and both lesions impair spatial working memory in radial-arm maze tasks (Aggleton et al., 1996; Sziklas & Petrides, 1999; Vann & Aggleton, 2003; Mitchell & Dalrymple-Alford, 2006; Vann, 2013, Harland et al., 2014; Nelson & Vann, 2014). The severity of spatial working memory deficits after ATN lesions reported here is reminiscent of studies on spatial alternation in the T-maze, albeit from different studies. That is, rats with ATN lesions generally produce severe impairments in T-maze tasks whereas MTT lesions often produce mild and sometimes transient deficits unless the availability of intra-maze cues is minimized (Vann & Aggleton, 2003; Vann, 2013; Aggleton & Nelson, 2015). On the basis of the current study, it seems unlikely that weaker impairments after MTT lesions in previous work was due to subtle procedural variations across studies.

The clear difference found between the effects of ATN and MTT lesions on spatial reference memory in the water maze, together with more similar effects in terms of their impact on spatial working memory in the same apparatus conflicts with the only previous report to examine the effects of MTT lesions on both measures (Winter et al., 2011). Closer inspection of that study, however, suggests that the impairment on spatial reference memory was at best mild and equivalent to controls by the final two of five days of testing. Winter et al. (2011) failed to find any deficit on spatial working memory after MTT lesions, but they used only two daily trials and the variable position of the platform was nonetheless constant relative to the pool wall, which means that rats may have used non-spatial strategies to solve

their task. Like the previous study to report impaired spatial working memory in the water maze (Vann & Aggleton, 2003) we used four daily trials and the distance between the platform and maze wall was varied when the position of the platform was changed across sessions. Inconsistent results have been found following mammillary bodies lesions, which have been reported both to disrupt (Sutherland and Rodriguez, 1989) and spare reference memory acquisition in the water maze (Santin, 1999). Unlike MTT lesions, ATN lesions consistently result in spatial reference memory deficits in the water maze, which are often reminiscent of the effects of hippocampal lesions (Moser et al., 1993; Warburton et al., 1999, Warburton & Aggleton, Wilton et al., 2001; Wolff et al., 2007; 2008). Spatial working memory deficits in the water maze after ATN lesions have also been reported previously (Van Groen et al., 2001).

A similar dissociation between MTT and ATN lesions to that reported here has been observed in a different water maze task that required the animal to learn a geometric discrimination to locate a fixed platform. On this task MTT lesions were able to discriminate the fixed location of a hidden platform at the same rate as controls whereas rats with ATN lesions remained at chance levels (Aggleton et al., 2009; Vann, 2013; Dumont et al., 2014a). Other evidence suggests that while short-term recognition of the location of familiar objects is impaired by MTT lesions (Nelson & Vann, 2014), the long-term acquisition of object-place paired associate learning is, unlike ATN lesions, completely unimpaired by MB lesions (Sziklas et al., 1996), and thus probably also unimpaired by MTT lesions. The long-term acquisition of spatial memory per se, however, is not intact after MTT lesions, because these lesions slow the acquisition of distal cues needed to discriminate two locations within a room (Nelson & Vann, 2014).

Together with other lesions studies, albeit separate comparisons of ATN and MTT lesions, the current findings reinforce the view that the ATN constitute a pivotal site within a complex array of inter-dependent structures now recognised as significant brain regions associated with episodic memory (Aggleton, 2008; Aggleton, Dumont & Warburton, 2011; Jankowski et al., 2013; Vann, 2013; Aggleton & Nelson, 2015; Dalrymple-Alford et al., 2015; Dillingham et al., 2015; Vann & Nelson, 2015). It seems likely that the key reason for greater, and more extensive, memory deficits associated with ATN lesions than with MTT lesions is that the ATN is a complex structure with a diverse and extensive set of neural connections. We know that lesions of the post-commissural fornix projection to the MB fail

to replicate the effects of MTT lesions (Vann et al, 2011; Vann, 2013). Together with the current findings, we consider it possible that direct and indirect connections between the ATN, the subicular cortex of the hippocampal formation, prefrontal cortex and retrosplenial cortex are responsible for the impairments associated with ATN lesions beyond the influence of the unique, unidirectional ATN pathway from the MTT (Aggleton & Nelson, 2015). It seems also unlikely that any one of these other multiple connections alone can be primarily responsible for all the effects of large ATN neuropathology. For example, an extensive study on the impact of fornix lesions on spatial learning tasks sensitive to ATN-hippocampal processes concluded that fornix pathways may contribute to tasks requiring flexible spatial and temporal cues, but that non-fornix pathways must contribute to spatial memory, particularly when tasks require more stable spatial solutions (Dumont et al., 2015). Based on neuroanatomical and electrophysiological evidence, strong arguments can be made that different components of the MB-MTT-ATN axis work together to support episodic memory and therefore factors that facilitate the acquisition of stable spatial solutions (Aggleton et al., 2010; Aggleton & Nelson, 2015; Dillingham et al., 2015). The problem for that scenario in explaining our current findings is that more severe neuropathology across all three components of the MB was produced by MTT lesions than was produced by ATN lesions, yet spatial reference memory was unaffected by MTT lesions. That is, a simple account of additive separate deficits across the different components of the MB-MTT-ATN axis, and perhaps for that matter after other ATN disconnections, may not be sufficient to account for memory impairments after diencephalic injury.

Many features beyond allocentric cues alone can factor in different spatial memory tasks. Although the use of allocentric cues may sit at the pinnacle of a hierarchy of strategies that rats use to solve spatial memory tasks, some tasks may also be influenced by moderating factors such as proactive interference and cognitive flexibility or be more susceptible to changes in alternate neural pathways that may compensate or exacerbate performance in any given spatial memory task. Particularly in view of the impact of MTT and ATN lesions on MB neuropathology, we conclude that our study instead lends support to Aggleton and Nelson's (2015) alternate proposal that ATN lesions give rise to a hierarchy of deficits and impact a variety of cognitive skills that explain the diverse pattern and severity of deficits produced by injury to this region.



## Chapter 7

# Object-place paired-associate learning

### 7.1. Introduction

A fundamental property of episodic memory is the ability to bind individual elements of an event such as learning arbitrary associations between stimuli and their context (Preston & Eichenbaum, 2013). Evidence from animal studies suggests that such paired-associate learning is dependent on the hippocampus and prefrontal cortex when the new association concerns individual items and their spatial or temporal context. For example, both hippocampal lesions and prefrontal lesions in rats or monkeys impair learning specific object-place and odour-place associations (Sziklas & Petrides, 1996; Gilbert & Kesner, 2002; Kesner & Ragozzino, 2003; Browning et al., 2005). By contrast, hippocampal lesions do not impair associative memory tasks involving non-spatial or non-contextual cues, such as object-odour pairings (Gilbert & Kesner, 2002). The current chapter addresses the question of whether MTT lesions impair the acquisition of object place associations.

The clinical relevance of associative memory tasks is that patients with the alcoholic Korsakoff syndrome are impaired when required to place an event or an item within a spatial context (Kessels et al, 2000; Kessels & Kopleman 2012; Kopelman, 2014). As mentioned previously, pathology in both the ATN and MB are consistently associated with the anterograde amnesia present in these patients, suggesting a role for the diencephalon in paired-associated learning (Kopleman, 2014; Aggleton, 2014). In one example, Pitel et al. (2008) found that Korsakoff patients performed significantly worse than non-Korsakoff alcoholics and healthy controls at learning arbitrary associations between words and spatial locations or temporal contexts.

Consistent with this perspective, ATN lesions in rats impair both object-place and odour-place learning (Sziklas & Petrides 1999; Gibb et al., 2006; Dumont et al., 2014). In their task, Sziklas & Petrides (1999) trained rats to learn that only one of the two objects was rewarded in the north location in an arena and the alternate object was rewarded in the south location. Rats with ATN, like rats with hippocampal lesions, performed poorly on this task

and showed no improvement even after 500 trials (Sziklas et al., 1996; Sziklas & Petrides 1999). Using a crossed-unilateral lesion preparation Henry et al. (2004) showed that interactions between the dorsal hippocampus and ATN were critical for acquiring these object-place pairings. In addition to spatial memory, the ability to form arbitrary associations between a stimulus and a context is a fundamental aspect of episodic memory processing (Kessel & Kopelman, 2012). The MB are critically implicated in this process within a clinical context and are thought to provide a unique brainstem influence, which contributes to ATN functioning and would be expected to disrupt object-place associations (Vann & Nelson, 2015). Surprisingly, rats with lesions to the mammillary body region have been reported to be unimpaired on the object place task using an arena, despite being severely impaired when tested for spatial working memory testing in the radial arm maze (Sziklas et al., 1996). This finding provides an anomaly within the literature and implies a very clear distinction between ATN and MB lesions, which therefore needs revisiting.

The effects of MB lesions should be replicated by MTT lesions the present study therefore re-examined the effects of MTT lesions to arbitrary object-place associations. MTT lesions should provide a selective removal of MB inputs to the ATN without resulting in substantial damage to adjacent structures. One possible explanation for the discrepancy between the effects of MB lesions and ATN lesions reported by Sziklas et al. (1996) and Sziklas & Petrides (1999) is that MB rats may have been able to use direction cues to solve the object-place task. That is, in their task rats were always released from one direction when the objects were presented the south end of the arena and the opposite direction when they were presented north end, so that the correct object could be associated with direction of approach as well as place. It is possible that there was some degree of lateral MB sparing in Sziklas et al. (1996) rats that may have aided performance. Consistent with this explanation, a recent study found that rats with MTT lesions could not discriminate between two spatial locations when direction was irrelevant (Nelson & Vann, 2014). To address this issue, a modified version of this task used a large diamond shaped arena, to permit the addition of a probe trial in which to test the influence of potential strategy use. Rats with MTT and sham lesion rats were trained to associate one of two visually and texturally distinct objects (presented simultaneously) as the rewarded object in one of two locations in the diamond arena. If rats with MTT lesions rely on directional information to acquire this task, then we would expect impaired performance on 'place' probe trials. In these trials the entire maze was

rotated by 90° to retain the same spatial location of the objects, but changes the direction of approach. Also, if the ability to use direction was a factor we would expect MTT rats to be less affected because previous studies have suggested that the projections of the lateral MB are probably intact after MTT lesions (Vann & Albasser et al., 2009). An additional ‘reversal’ probe test in which rats were run from the opposite starting point from where they were trained was used to ensure rats were not using egocentric cues to discriminate between objects. For this probe we would expect rats to be unimpaired if they are using direction cues to solve the task, but below chance if they are relying on egocentric (body turn) information. In a final probe both objects were baited to ensure rats were not using local odour cue to select an object.

## 7.2. Method

Following water maze testing described in Chapter 6 the MTT lesion and MTT sham lesion rats were placed on food restriction and given a 12 day rest from testing until their body weights were approximately 85% of their ad libitum feeding weights. They received 7 days of habituation and pre-training for the object-place association task. During this period the ATN and ATN sham lesion group were receiving additional water maze testing as part of a parallel ATN lesion enrichment study.

The paired-associate task was examined in a large, open diamond-shaped enclosure (200cm wide 150cm long at the points of the diamond shape). Both the north and south ends had a recessed start chamber that could be blocked off from the rest of the enclosure by a guillotine door and the east and west corners had recesses to allow the insertion of two large complex objects (figure 7.1a and 7.1b). The apparatus was placed ~1m above the floor in a windowless room that contained salient extra-maze cues such as a computer, tables, posters, curtains etc. The rats were divided into two groups and each group was only ever trained using entry into the arena from the north or south start chambers. The east or west corners of the diamond were used to present the objects as an adjacent pair that alternated left and right local positions pseudo-randomly across trials. One object was a cube covered in smooth pebbles and the other a star shape covered in studded rubber matting (figure 7.1a). Each object was approximately 12cm x 12cm x 12 cm and had an opening facing the arena that was large enough for a rat to put its head in to retrieve a food reward (two 0.1g chocolate drops) from a recessed food well (6cm × 7cm × 2 cm) which was located immediately inside. Both food wells were also baited with inaccessible food to provide constant odour cues.

For this task one of the pair of objects was correct (irrespective of specific local position) when it was located in the east corner of the maze and the alternate object was correct when located in the west corner of the maze. The correct object-place associate-pairing was counterbalanced between the two groups of rats, so that the correct object in one location for one group was the incorrect object in that location for the other group and vice versa for the opposite location. During pre-training both objects were baited with ~6 chocolate pellets, and chocolate pellets were scattered in front of the objects to encourage the rats to approach and explore them. During formal testing each rat received six daily trials in which the two objects were presented in either the east or west corner of the maze on 3 of the daily trials each. The object presentations were counterbalanced in a pseudorandom sequence so that each rat received equal trials with the objects in the east and west corners. In addition the local placement of each object within a corner was counterbalanced so the rats did not just learn to select an object based on its relative distance or position from the start chamber. Rats were trained to a completion criteria of 21 correct choices out of 24 trials across 4 days up to a maximum of 40 days, with no less than 4 correct choices on any one day. At the start of a trial the rat was placed in the start chamber with a closed guillotine door. The door was then lifted to allow the rat to explore the diamond enclosure. A trial concluded when the rat inserted its head and forepaws into either of the two objects. The rat was then removed and returned to a holding cage, so that rats were run in squads of 3-4 with an inter-trial interval of approximately 3-4 minutes. Once the rats had reached criterion they were tested on a series of three probe sessions each consisting of 6 trials to examine strategies used to perform the task.

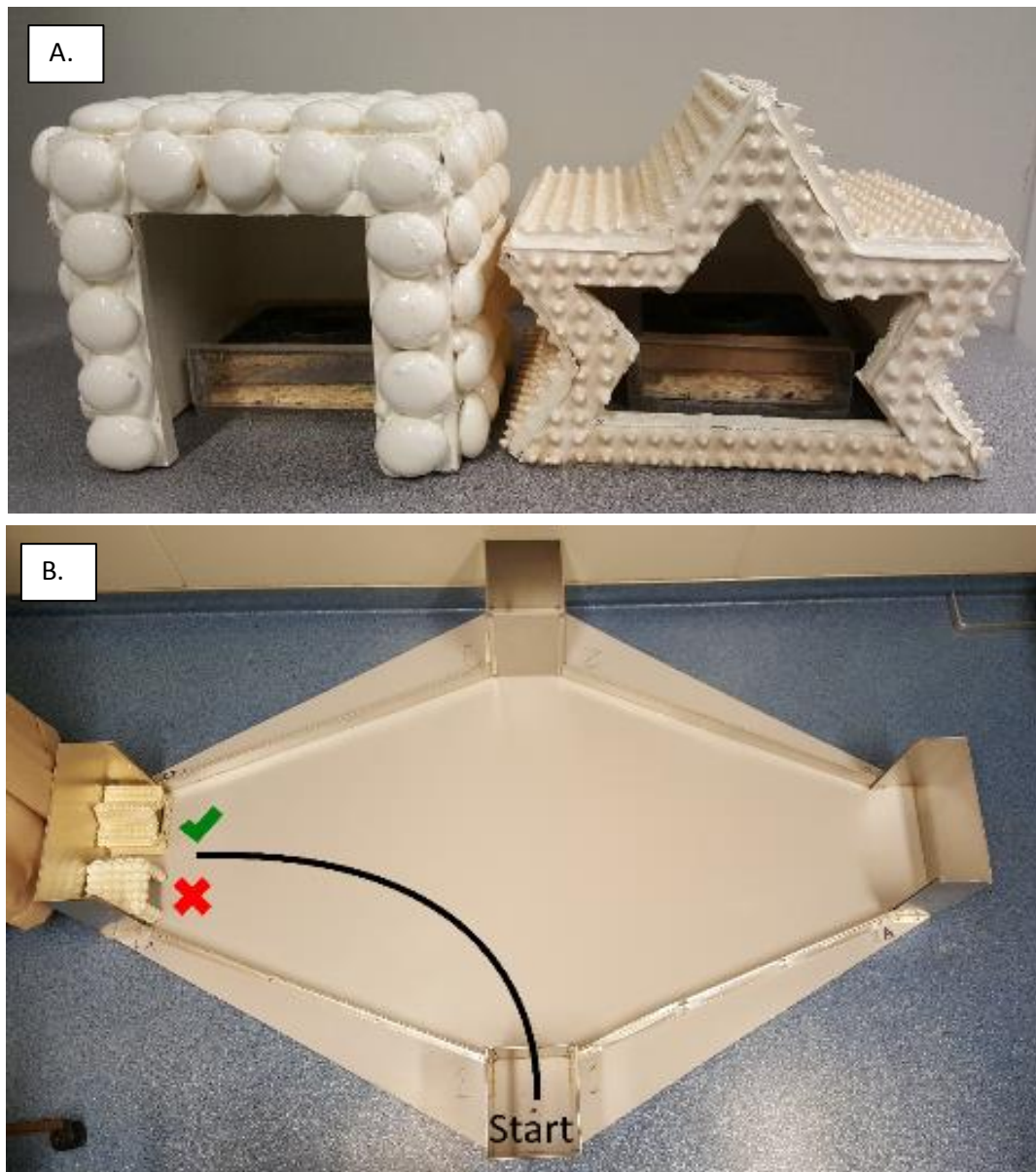


Figure 7.1: Images depicting (A) the two complex objects and (B) the diamond arena used for the object-place paired-association task. Only one of the two objects was correct when presented in east corner of the diamond and the alternate object correct when presented in the west corner of the diamond (left and right recessed areas in B).

### 7.2.1 Three probe tests

For the first probe (Double baiting probe) both objects were baited with chocolate drops in all six of the day's trials to ensure that the rats were not using local odour cues to select the correct object.

For the second probe (Reversal probe, figure 7.2a) the rats were run from the opposite release point from where they had been trained to determine whether they were relying on body turn (or egocentric) information to do the task i.e. turn left for object A is correct, turn right when object B is correct.

The third probe (Place probe, figure 7.2b) examined whether rats relied on directional information or spatial location to identify the correct object. Thus in the final probe session the entire maze was rotated 90 degrees and the opposite starting door was used so the rats could not use either body turn or directional information about the room, and instead would have to rely on the association between the object and its location independently of start location. For all six of these trials the objects were only placed in the "east" side of the maze (same location relative to room cues as before) and the local position of the object was again randomised so the rat received three trials with the object closest to the start door and three where it was slightly further away.

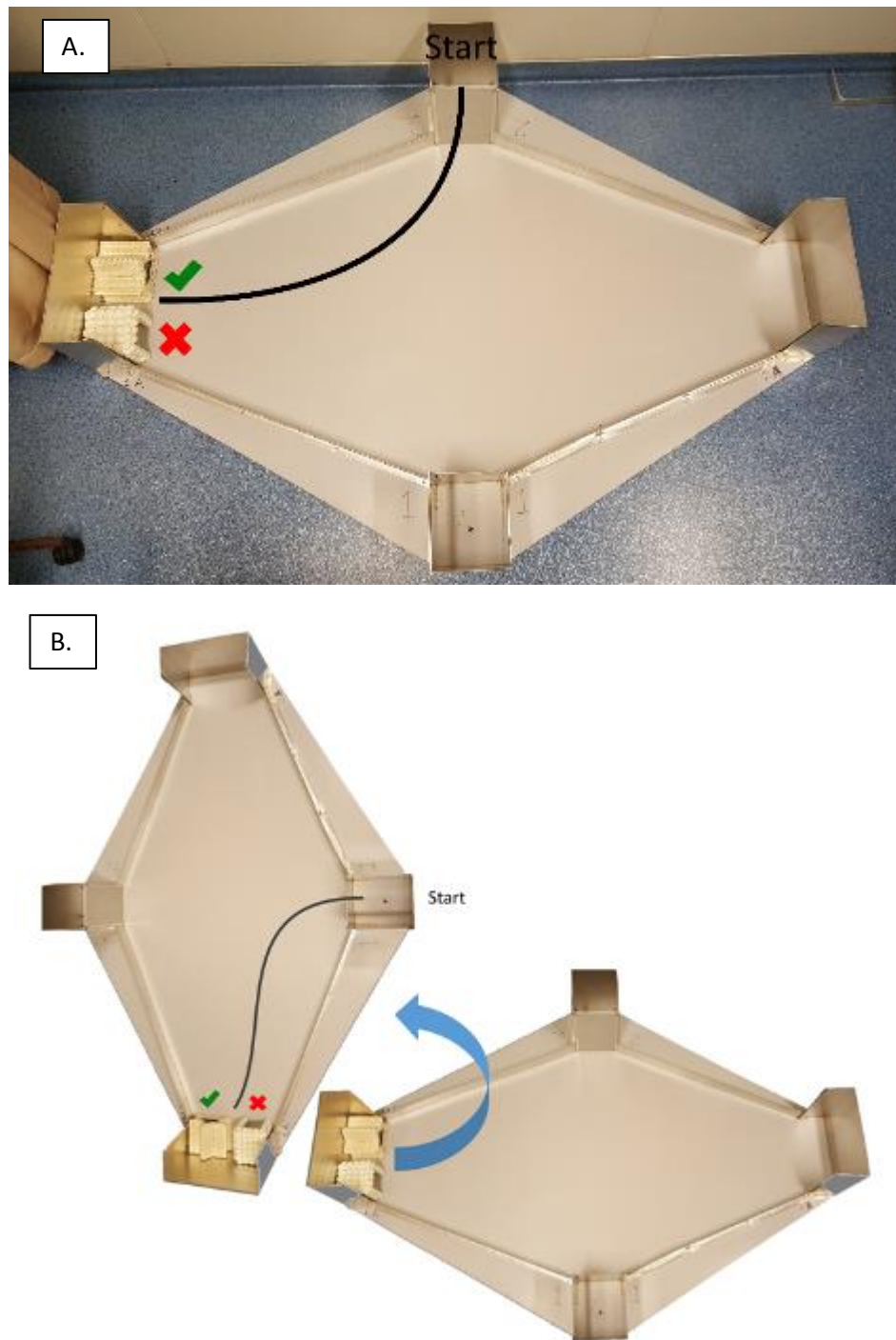


Figure 7.2: Images depicting (A) the reversal probe and the place probe (B). In the opposite start probe the rats were run from the opposite start door than used during training. For the place probe the entire maze was rotated by 90 degrees so that the location of the objects relative to room cues was the same, but rats could no longer use directional cues.



## 7.3. Results

### 7.3.1. Acquisition of object-place paired association

The 40 days of testing on the object-place paired association task were summarised as eight five-day blocks and the mean percentage of correct trials for each group was analysed with an ANOVA using block as a repeated measure. As shown in figure 7.3 both groups required extensive object place pairings (120 + trials) before they were able to discriminate above the chance level. Rats with MTT lesions acquired the task at a slower rate (Lesion ( $F(1,21) = 12.66, p < 0.01$ ) and did not reach the level of accuracy shown by the sham group in the final blocks of testing (Block x Lesion ( $F(7,147) = 4.51, p < 0.001$ ). Nonetheless, both groups clearly improved their performance with training (Block ( $F(7,147) = 35.13, p < 0.001$ ) suggesting that MTT lesions impair the acquisition of object-place associations but do not preclude them altogether.

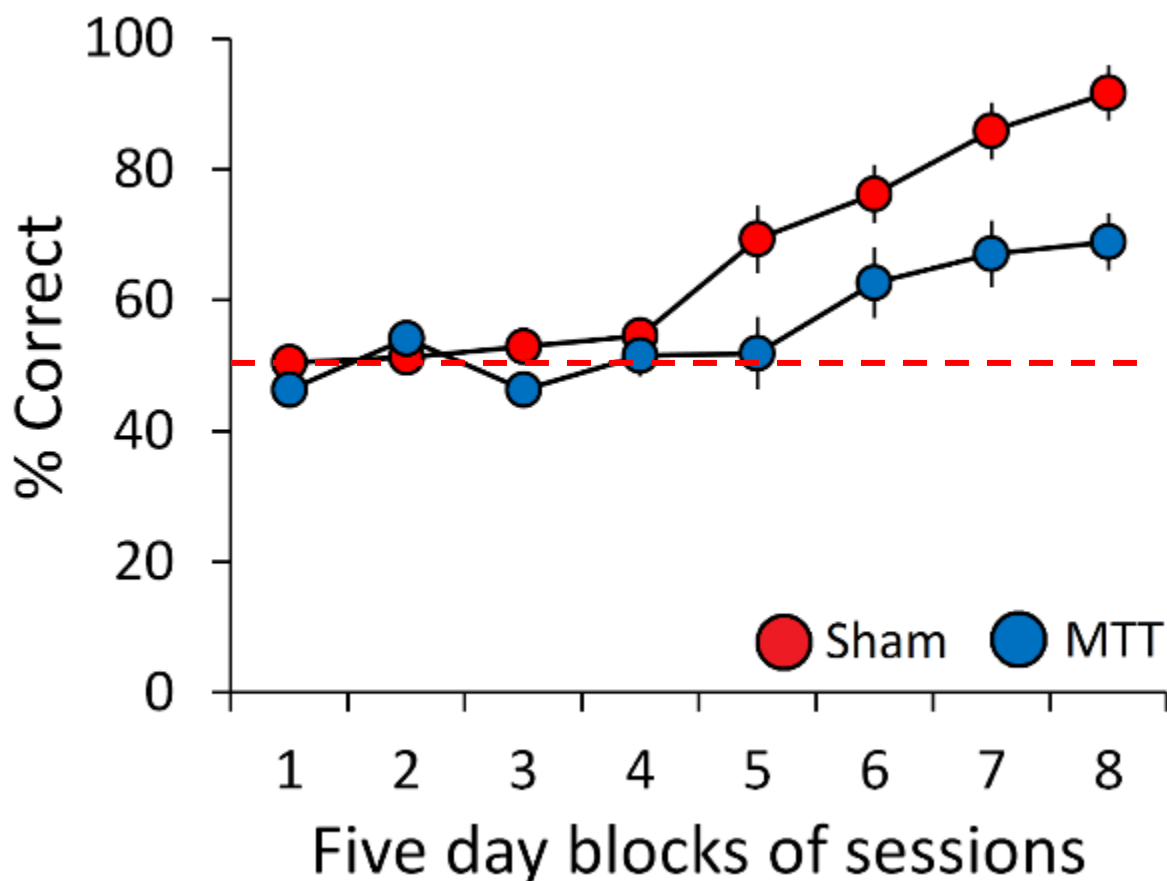


Figure 7.3: Mean  $\pm$  SEM percentage of correct object-place pairings over blocks of trials (5 days per block) in the object-place paired-association task. The red dashed line indicates chance level performance.



### 7.3.2. Double baiting probe

In the first probe test, both objects were baited with chocolate drops to ensure that the rats were not relying on local odour cues to select the correct object. Both groups performed well during this probe test although the MTT lesion rats were still impaired relative to shams ( $t(1,21) = 2.27, p < 0.05$ , figure 7.4). Hence it appears that neither group relied on reward-related olfactory cues to discriminate between the two objects.

### 7.3.3. Reversal probe

On the following day the rats were run from the opposite start door to determine whether they were relying on egocentric information (body turn when exiting the start chamber) to discriminate between objects. Here, MTT lesion rats were again impaired relative to sham rats ( $t(1,21) = 3.83, p < 0.001$ , figure 7.4) and both groups performed at a similar level to that shown during the end of acquisition.

### 7.3.4. Place probe

The final probe was used to control for directional information in addition to egocentric strategies, as the particular direction within the room could determine object selection even when opposite start positions are used. For this probe the maze was rotated 90° so that one of the object-place pairings retained its relative location to general room cues, but the direction to reach the objects had changed. As for the previous probes the MTT lesion rats were impaired relative to the shams ( $t(1,21) = 3.06, p < 0.01$ , figure 7.4) which were very accurate across the 6 trials. Together these three probe trials suggest that rats were primarily relying on the object-place pairings to discriminate between the objects.

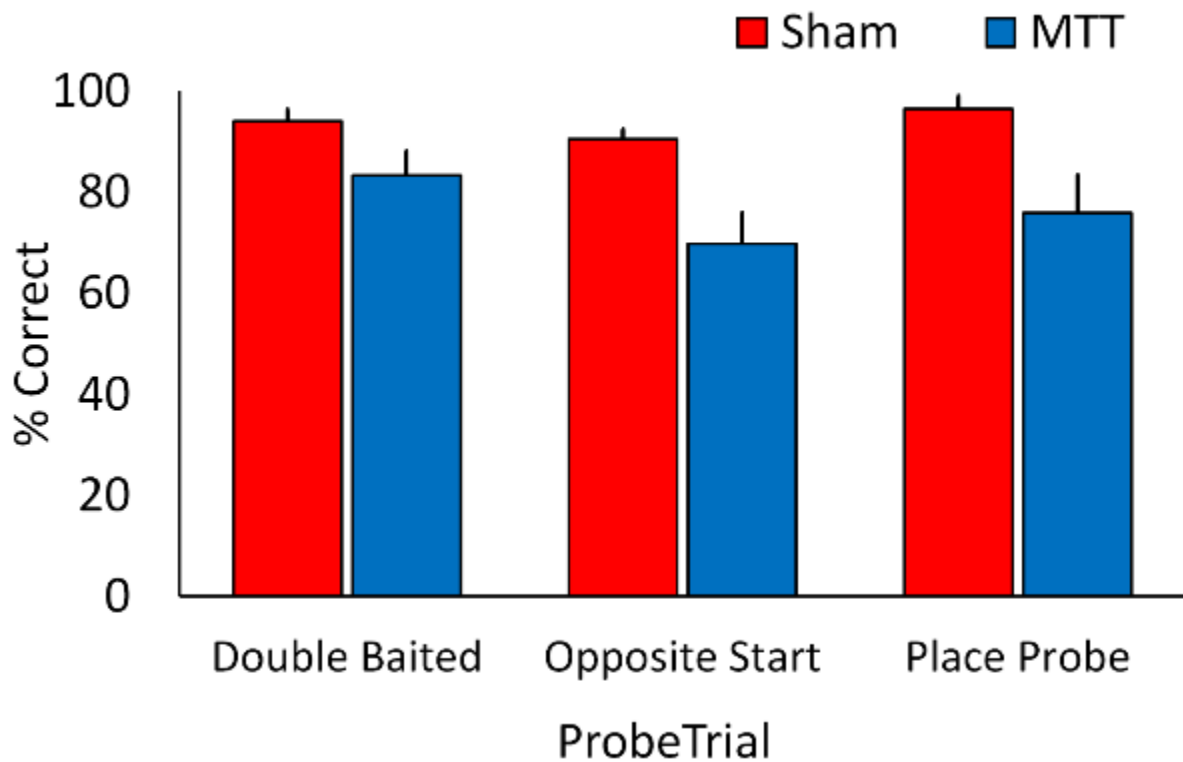


Figure 7.4: Mean + SEM percentage of correct object-place pairings for the three probe trials used to determine discrimination strategies used by each group.

#### 7.4. Discussion

The current study resolved a discrepancy between the effects of ATN and MB lesions on an object-place paired associate task reported in the literature (Sziklas et al., 1996; Sziklas & Petrides, 1999). The present findings provide convincing evidence that MTT lesions impair acquisition of arbitrary object-place pairings. The ability to form arbitrary associations between a stimulus and a context is a fundamental aspect of episodic memory processing (Preston & Eichenbaum, 2010). None of the three probe trials employed disproportionately affected either group, suggesting that both groups relied primarily on spatial, not egocentric or directional information to discriminate between objects. MTT lesions were used here to provide a selective removal of the MB influence to the rest of the extended hippocampal circuit. Previously MB lesions have often included damage to adjacent structures and pathways (Sziklas et al., 1996; 1993, but see Vann & Aggleton, 2003).

Consistent with the current findings a recent experiment found that rats with MTT lesions impaired discrimination on a one trial object-in-place spontaneous recognition test

(Nelson & Vann, 2014). In this study MTT lesions also impaired acquisition of a place (only) discrimination when directional information was irrelevant (Nelson & Vann, 2016). Recent evidence has emphasised the importance of MB inputs and strongly suggests that rather than a passive hippocampal relay, the MB provide a unique brain stem influence to the extended hippocampal circuit (Vann, 2009; Vann et al., 2010; Vann, 2013). The importance of this influence to ATN function has been emphasised in the literature, but the dense reciprocal connections between the ATN and the hippocampal formation offer an alternative pathway that might otherwise explain the difference between ATN lesions and MB lesions on object-place associative memory (Dillingham et al., 2015; Jankowski et al., 2013).

Using a task similar to that used here, Sziklas et al. (1996) reported that rats with large electrolytic lesions to the MB region, or lesions to the fornix, acquired object-place pairings at an equivalent rate to controls (Sziklas et al., 1996; Sziklas et al., 1998). In their task, rats were required to learn that one of two different objects was rewarded in one end of an open arena and the alternate object was rewarded when the pair was presented at the opposite end of the arena. The lack of deficit observed on their task did not appear to be due to spared spatial processing because the same rats with MB or Fornix lesions were severely impaired on a standard RAM task (Sziklas et al., 1996; Sziklas et al., 1998). One possible explanation for the disparity between the present finding and those of Sziklas et al. (1996), is that the MTT lesions provided a more complete and selective removal of the MB inputs to the ATN, but this seems unlikely given the size and extent of their lesions. It is possible that slight methodological differences such as the shape and size of the objects and arena might account for these disparate findings, which could only be addressed by direct within-study comparison between.

Although, the acquisition of object-place associations was impaired following MTT lesions, by the final block of testing this group was discriminating significantly above the level of chance. It is possible that with extended training the MTT lesion group may have shown further improvement. By contrast, both ATN and hippocampal lesions produced a severe and persistent impairment on a similar task showing no evidence of improvement even after 500 trials (Sziklas et al., 1996; Sziklas & Petrides, 1999). The results of two crossed unilateral lesion studies suggest that the ATN and hippocampus function inter-dependently to support the acquisition of these object-place pairings (Henry et al., 2004; Dumont et al., 2010). Furthermore, the addition of a retrosplenial cortex to the hippocampal lesioned

hemisphere produced equivalent impairment to bilateral hippocampal lesions (Dumont et al., 2010). To explain these findings, Sziklas et al. (1999) suggested that the comparable effects observed after lesions of the ATN or the hippocampus on the object-place paired associate task might relate to their shared dense connections with the retrosplenial cortex. Therefore intact ATN-retrosplenial-hippocampal connections may explain why our MTT lesions produced less severe effects than those previously reported for ATN lesions.

Recent evidence has also suggested that MTT lesions impair recency judgements about a list of objects (Nelson & Vann, 2016). This finding is of particular note because impaired temporal context memory is a common finding in patients with the Korsakoff syndrome (Kressels & Kopelman, 2012; Kopelman, 2014). Previous work has also shown that ATN lesions impaired temporal order discriminations for a list of objects (Dumont & Aggleton, 2013) and odours (Wolff et al., 2006). The latter finding is consistent with deficits found after explicit hippocampal lesions (Fortin et al., 2002). Thus far, behavioural studies of MTT lesions have relied heavily on visual and spatial information (Dillingham et al., 2015). However, to determine the breadth of MTT lesion deficits it is necessary to examine the effects of MTT lesions on tasks without explicit visual/spatial demands, such as the odour recency task (Fortin et al., 2002).

The results of this study provide direct support for that the MTT, and by inference also the MB, contributes to the acquisition of arbitrary associations between an object and a spatial location. However, the deficit reported here does not appear to be as profound as those previously observed for ATN or hippocampal lesions (Sziklas et al., 1996; Sziklas & Petrides, 1999). While the effects of ATN lesions were not examined in the current study, it suggests another line of evidence to extend those reported in chapter 6 that MTT lesion effects are generally milder than those of ATN lesions. These findings provide further evidence that the dense reciprocal connections between the ATN and hippocampal formation, especially via the retrosplenial cortex, might be especially relevant for forming object-place associations.

## Chapter 8

# MTT lesions and rhythmic oscillatory activity.

### 8.1. Introduction

Previous chapters have demonstrated that episodic memory is supported by a distributed neural network in the brain, which includes the hippocampal formation (HPC), retrosplenial cortex (RSC), prefrontal cortex (PFC), anterior thalamic nuclei (ATN), mammillary bodies (MB) and interconnecting fibre tracts, the fornix and the mammillothalamic tract (MTT) (Aggleton & Brown, 1999; Aggleton, 2014; Aggleton & Nelson, 2015; Dillingham et al., 2015; Dalrymple-Alford et al., 2015). Within this circuit, the MTT was noted as being of considerable interest because it is consistently implicated in anterograde amnesia following thalamic stroke (Van der Werf et al., 2000, 2003; Carlesimo et al., 2011). Furthermore, the MTT is unique in being a fibre path that appears to be solely involved in the extended memory circuit, together with conveying the brainstem influence of the ventral tegmental nuclei of Gudden (VTg) via the MB (Vann, 2009; Vann, 2013). Moreover, the earlier chapter also confirmed, using zif268 immunostaining, that MTT lesions produce functional alterations in terms of IEG deficits in cortical and subcortical regions, which may contribute to the lesion deficit (Vann & Albasser, 2009; Vann, 2013; Frizzarti et al., 2016). These changes, however, have been examined post-mortem in isolated structures, which provides limited insight into the altered or dysfunctional interactions across the neural network during task-related information processing.

One notable feature of the medial MB is that they are known to propagate rhythmic oscillatory activity, via the MTT, to the ATN and subsequent upstream brain structures (Vann & Aggleton, 2004). Therefore, it would be informative to examine rhythmic interactions between the HPC, ATN and PFC following MTT lesions. As discussed in chapter 5 within neural networks the cumulative electrical activity of all local membranes, primarily from synapses, gives rise to an extracellular field potential (Buszaki et al., 2012). This activity often oscillates in waves of varying frequency and amplitude ranging from slow delta activity (1-4 Hz) to fast gamma activity (30-100 Hz). Synchronisation, or coherence, of this rhythmic activity between brain structures provides a platform for the inter-regional communication

necessary for complex cognitive functions such as memory (Colgin, 2011; Fell & Axmacher, 2011). Two frequency bands in particular, theta and gamma, have been consistently associated with mnemonic processes in both humans and animals. Theta rhythm is a low frequency oscillation (4- 8 Hz in humans, 8-12 Hz is alpha; 4-12 Hz in rats) that is associated with synchronising distally located neural networks in the brain such as the hippocampus, diencephalon and limbic cortex (Kirk & Mackay, 2003, Colgin, 2011, Ketz et al., 2015). By contrast, fast oscillations like gamma (30-100 Hz) are suggested as being more involved in local processing and are thought to represent discrete units of information (Jensen & Colgin, 2007). When combined, in a process known as cross-frequency coupling, the distinct properties of these two frequency bands have been suggested to form a mechanism to encode and temporally order memory representations (Jensen & Colgin, 2007).

There is a vast literature exploring the electrophysiological correlates of successful memory performance in humans. The majority of these studies have relied on delayed recall or recognition paradigms for words or visual scenes and then examined electroencephalogram (EEG) recordings from scalp electrodes (Nyhus & Curran, 2010). Across EEG studies, both theta and gamma phase synchronisation are associated with the successful encoding and retrieval of episodic memories (Nyhus & Curran, 2010; Colgin, 2011; Colgin, 2013). Such studies have consistently reported that an increase in cortical theta coherence in healthy controls, and cortical-hippocampal interactions in epileptic patients, was predictive of successful memory processing (Kilmesch et al., 1997; Weiss et al., 2000; Mölle et al. 2002; Nyhus & Curran, 2010). Taking a different approach, Mormann et al. (2005) explored the separate roles of theta and gamma oscillations using intracranial recording from the medial temporal lobe in patients with epilepsy. This study found that memory encoding and retrieval in the perirhinal cortex and hippocampus was associated with increased gamma activity, whereas theta served to facilitate interactions between the two regions during a memory task (Mormann et al., 2005). Recent evidence has further emphasised the importance of theta-gamma interactions for successful memory performance. Heusser et al. (2016) used an episodic sequence memory task and found that items in a sequence were represented by peaks in gamma power along distinct phases of an underlying theta oscillation. Moreover, this segregation of gamma waves was related to successful temporal order memory (Heusser et al., 2016). These studies provide strong evidence for the involvement of theta and gamma oscillatory activity in memory processes by allowing temporal specificity between task onset

and electrical activity. In addition to the considerable clinical evidence, animal studies have reinforced the critical role of theta and gamma rhythmicity in learning and memory processes.

Rodent studies have recapitulated the importance of theta and gamma oscillatory activity in successful memory processing. This literature has focused heavily on the hippocampal formation and prefrontal cortex, with little consideration given thus far to rhythmic activity within the diencephalon. Because of its ubiquity throughout the hippocampal formation, theta activity has received the greatest attention in rodents (Buzsaki, 2002). Compelling evidence directly implicating hippocampal theta in mnemonic processes was provided by McNaughton et al. (2006). In this study, inactivation of the medial septum was used to abolish hippocampal theta. This resulted in severely impaired spatial memory in the water maze. However, when hippocampal theta was reinstated via a bypass circuit from the supramammillary nucleus, memory performance returned towards baseline levels (McNaughton et al., 2006). There is also considerable evidence that coupling of rhythmic activity between the hippocampus and prefrontal cortex, especially in the theta band, is fundamental to the acquisition and maintenance of spatial memories in rodents (Colgin, 2011, 2013). Increased theta coupling between the hippocampus and prefrontal cortex during a period of high cognitive load or a correct trial has been consistently reported in rats performing a spatial working memory task (Jones et al., 2005; Hyman et al., 2010; O'Neill et al., 2013). Furthermore, the importance of HPC-PFC coupling does not appear to be restricted to working memory tasks. Kim et al. (2011) found that neurons within the medial prefrontal cortex became increasingly phase-locked with hippocampal theta oscillations as rats learnt an object-place pairing.

Rodent studies have also provided support for a mnemonic role of theta-gamma interactions within the hippocampus. For example, Tort et al. (2009) found that an increase in accuracy on an object-place paired associate task was paralleled by the strength of theta-phase gamma-amplitude coupling in hippocampal CA3. Similarly, Shirvalkar et al. (2010) reported that the strength of theta-gamma co-modulation, which is the simultaneous increases in the amplitude of each frequency band, in the hippocampus was able to predict performance on a single trial spatial working memory task the water-maze version of the RAM. Clearly, both human and rodent evidence suggests an important role for rhythmic oscillatory activity in successful memory processing in the intact brain. However, substantial insight in to

memory might also be gained by examining the evidence for dysfunctional rhythmic activity in neurodegenerative and neuropsychiatric diseases associated with impaired memory in humans.

Aberrant rhythmic activity has been associated with various neurological diseases and likely contributes to impaired cognition in these conditions (Basar et al., 2016; Palop & Mucke, 2016). Abnormal changes in the strength, occurrence and coherence of rhythmic activity in neurodegenerative diseases have been proposed to reflect the loss of neuromodulatory functions and specialised interneurons. The loss of these mechanisms impacts the recruitment of networks of neurons at the specific frequencies necessary for highly complex cognitive functions such as memory (Nimmerich et al., 2015). For example, an abnormal increase in the strength of low frequency oscillatory activity is observed in patients with Alzheimer's disease (AD), Parkinson's disease (PD) and schizophrenia (Hermann & Demiralp, 2005; Burke et al., 2013; Sun et al., 2013; He et al., 2016; Nimmerich et al., 2015). Critically, diencephalic pathology has been associated with cognitive impairments present in all of these conditions suggesting a possible link between diencephalic damage and changes to rhythmicity (Braak & Braak, 1991a,b; Delacourte et al., 1999; Young et al., 2000; Rub et al., 2002; Aggleton et al., 2016). EEG recordings from patients with AD have reported global reductions in gamma power, which are thought to be indicative of pathological activity in the cortex (Hermann & Demiralp, 2005). Furthermore, increased theta and decreased alpha power (slowing of oscillatory activity) at temporal and parietal sites were found to be the best predictors of which patients with mild cognitive impairment (MCI) would progress to AD over a 21 month period (Jelic et al. 2000). In a more recent study, progression to AD over 2 years was accurately predicted by decreased alpha power and coherence in the cerebral cortex, especially above the posterior cingulate (Prieto del Val et al., 2016). Like AD, abnormal increases in slow wave oscillations have been observed in cognitively impaired patients with PD. In one study, He et al. (2016) found that PD patients with and without MCI could be differentiated based on occipital, temporal and frontal theta activity, with increased theta activity found in patients with MCI. Furthermore, deficits in attention, visuospatial and executive functions in the PD-MCI group were found to correlate with theta power in the frontal cortex (He et al. 2016). Like AD, patients with schizophrenia were found to have reduced gamma power, but only during sensory encoding (Sun et al., 2013). Increased activity within the default mode network in



lower frequency bands, such as theta has also been observed in schizophrenia (Burke et al., 2013).

These disease related changes have been further elaborated in animal models, which replicate critical pathological characteristics of a disease (Palop & Mucke, 2016). In one study, transgenic mice overexpressing human amyloid precursor protein, a hallmark of AD neuropathology, had profound reductions in cortical gamma amplitude, which was associated with profound spatial memory deficits in the water maze (Verret et al., 2012). Furthermore, there is animal evidence that changes in electrophysiological activity might even pre-empt the appearance of amyloid beta pathology. Goutagny et al. (2013) found reduced theta high-gamma cross frequency coupling in the subiculum prior to the appearance of neuropathology in transgenic AD mice. Furthermore, in a maternal immune activation model of schizophrenia, HPC-PFC coherence was observed to be profoundly reduced across the frequency spectrum (Dickerson et al., 2010). The aforementioned research suggests that altered of dysfunctional rhythmic oscillatory activity, especially within the theta and gamma bands, is associated with and can predict profound cognitive impairment. Moreover, the distributed nature of these changes, not only in terms of electrophysiology, but also neuropathology, emphasises the need to move beyond single structures to gain a more balanced understanding of the neural substrates of memory (Aggleton et al., 2016). As discussed in chapters 3,4 there is a plethora of evidence suggesting that damage to the ATN-MTT-MB axis, consistently results in profound memory impairments and functional alterations throughout the extended hippocampal system, likely contributing to the behavioural deficits. Rat studies have revealed that the hippocampus and diencephalon function interdependently during spatial memory tasks (Warburton et al., 2001; Henry et al., 2004; Dumont et al., 2010). However, the exact nature of these interactions is not well-defined and greater insight into the underlying neural representation of memory may come from determining how damage to these structures impacts the interactions between the two primary memory regions, the hippocampus and PFC.

One possibility is that rhythmic oscillatory activity propagated by the diencephalon functions to temporally coordinate activity between the diencephalon, neocortex and hippocampus during mnemonic processes (Kirk & Mackay, 2003; Ketz et al., 2015). Consistent with this proposal coherent theta activity is present throughout the limbic diencephalon, neocortex and hippocampus, which strongly suggests the presence of

integrated information processing (Bland et al., 1995; Kirk et al., 1996; Kocis and Vertes, 1994; Vertes et al., 2001; Buzsaki, 2002; Talk et al., 2004; Tsanov et al., 2011 a,b). Furthermore, a recent clinical study found that theta coherence and theta-gamma cross-frequency coupling between cortical scalp electrodes and intracranial ATN electrodes increased during encoding and was able to predict later recall of visual scenes (Sweeney-Reed et al., 2014). Recent evidence has also suggested that the diencephalon is capable of modulating activity in the hippocampus and prefrontal cortex. For example, stimulation of the ATN can modulate LFP in the HPC and increase a BOLD response in the HPC and PFC (Stypolkowski et al., 2014; Gibson et al., 2016), increase neurogenesis in the dentate gyrus and aid performance on memory tasks (Encinas et al., 2007; Toda et al., 2008; Hamani et al., 2011). However, recent findings suggest that the inputs to the MB from the VTg, and not the hippocampus appear to be critical for spatial memory function in rats (Vann, 2009; Vann et al., 2011; Vann, 2013). This is of particular note because the VTg provides a unique theta input, suggested by the authors to be a possible “pontine hippocampal theta generator” (Bassat & Poindessous-Jazat, 2001). If this is the case, rather than merely coordinating synchronised activity between structures in the extended memory circuit, MB inputs could be expected to actively modulate hippocampal activity. A critical question then, is whether MTT lesions disrupt rhythmic activity within the extended hippocampal circuit and whether this disruption is present during spatial memory processing.

The aim of the experimental work in this chapter was to investigate the impact of MTT lesions on rhythmicity both within and across the extended circuit, using electrophysiological recordings taken in the ATN, PFC and dorsal hippocampus. MTT lesions provide a selective and more complete means of removing the MB/VTg theta input in to the ATN, than explicit lesions to the MB (Dillingham et al., 2015). The loss of MB theta is thought to contribute to the memory deficits associated with MTT lesions, but the impact of MTT lesions on rhythmic activity within and across the system has not yet been examined. This contention is supported by evidence that MTT lesions produce altered expression of neural activity markers across the extended hippocampal network suggesting dysfunctional interactions might be taking place (Vann & Albasser, 2009; Vann, 2013; Frizzarti et al., 2016). Rhythmic interactions between the ATN, HPC and PFC would be expected to be at a maximum during mnemonic processing especially at critical decision points within a maze (Kirk and Mackay, 2003; Colgin, 2013). To address these issues rats were given MTT or

sham lesions and subsequently implanted with microwire electrodes in the HPC, ATN and PFC. Electrophysiological signals were recorded throughout spatial working memory testing in the radial arm maze, which allowed epochs of low cognitive load (the first four arm choices) to be compared with epochs of high cognitive load (the last four arm choices).

In the second part of this experiment investigated the efficacy of ATN theta burst stimulation (TBS) to enhance memory performance in behaving rats. ATN stimulation has been shown to result in improved cognitive performance in humans and rats and modulated activity in the hippocampus and prefrontal cortex (Oh et al., 2012; Hamani et al., 2011; Stypulkowski et al., 2014; Gibson et al., 2016). The electrophysiological impact of ATN TBS-stimulation on the hippocampus and prefrontal cortex was first assessed in a standard cage, before being applied to rats during their first 4 arm choices in the RAM. At the end of the experiment *zif268* activity was driven by unilateral stimulation of the ATN, so stimulation related *zif268* changes could be examined across hemispheres in the hippocampus and retrosplenial cortex.

## 8.2. Method

### 8.2.1. Subjects

Subjects were 30 male PVGc hooded rats bred in-house and were between 8 and 10 months and weighed between 290-350g at the time of lesion surgery. The rats were randomly allocated to either MTT or sham surgery groups. All rats were housed in standard conditions of three or four rats per opaque plastic cage (50 cm long by 30 cm wide by 23 cm high), with reversed lighting conditions (lights off from 8am to 8pm) during which all behavioural testing was conducted. Following surgery all rats were housed individually for a recovery period of approximately 7 - 12 days. Food and water were available *ad libitum* during surgery, recovery and the initial behavioural tasks. Pre-lesion surgery training and later behavioural tasks required the rats to be deprived to 85% of their free feeding body weight, but water was still available *ad libitum*. All procedures complied with the University of Canterbury animal ethics guidelines and were subject to AEC approval.

### 8.2.2. Radial arm maze

This electrophysiology experiment required rats to be plugged into a headstage cable. For this reason a custom made radial arm maze was built with guide frames, pulleys and doors located beneath the top surface of the maze (70cms off the floor; figure 8.9) The clear Perspex guillotine doors (28cm high by 10cms wide) could be raised and lowered from the outer perimeter of the central hub (34cms across) to block access to the arms (65cm long by 10cms wide with a 5 cm high edge along each side). A clear Perspex barrier extended (30cms long by 25 cms high) down one side of the arm to prevent the rat from jumping directly across arms. The top corner of this barrier was rounded to allow the headstage cable to move freely. The entire maze was painted light grey and was mounted on a large square base (1m by 1m) with a freely rotating joint made from a PVC pipe. A series of pulleys located at the top and bottom of the door frames and on the base of the maze enabled the doors to be manually raised (arm closed) or lowered.

### 8.2.3. Habituation

Three weeks prior to habituation rats were put on food restriction. During this period the rats were also handled and habituated to the chocolate drops (0.1g) that served as a reward for this task. Prior to lesion surgery the rats were habituated to the radial arm maze for seven days. For the first three days cage groups were placed in the maze for 10 minutes with chocolate drops scattered lightly in the central hub and more densely down the arms and in the food wells. Then rats were habituated individually for three minutes a day with the doors raised up and down at random intervals. The chocolate drops were moved further down the arms so that by the fourth day of individual habituation the drops were present only in the food wells. Unlike the previous experiment, the rats were not trained preoperatively in the maze.

### 8.2.4. MTT lesion surgery

MTT lesions were made in the same manner as detailed in experiment 1 (chapter 6) with minor alterations to improve accuracy. The temperature of the radiofrequency probe was increased from 63° to 65°C and the dorso-ventral coordinate lowered from -0.71cm from dura to -0.72cm from dura.

### 8.2.5. Post lesion RAM testing

Following a recovery period of 7-10 days the rats were returned to the RAM for 12 days of standard work memory testing to examine lesion patency so that any rats suspected to have

incomplete lesions could be removed. This task was performed in the same manner as described previously (see chapter 6). Briefly, the rat was placed in the central hub of the maze with guillotine doors blocking access to the eight baited arms. At the start of the trial all doors were lowered and the rat was allowed to enter an arm. Once the rat had selected an arm, the door was raised to confine it to the arm for ~10sec regardless of outcome. The rat was then allowed to return to the centre hub and the sequence was repeated until all eight arms had been visited, the rat had made 20 arm visits or 10 minutes had elapsed.

### 8.2.6. Recording and stimulation electrode fabrication

Bundles of four wires were used to record local field potentials bilaterally from the AV region of the anterior thalamic nucleus and dorsal hippocampus, with a unilateral electrode in the prefrontal cortex. Four wires were used per electrode in this study to provide additional strength to the implant and to allow selection of the most patent channel for electrophysiological signals from each structure. Electrodes were constructed by twisting together four 50µm platinum iridium wires (90% platinum, 10% iridium) which had a heavy polyamide coating (California Fine Wire, CA USA). The wires were then fused by applying heat from three different directions with a heat gun and then sealed with a thin coating of cyanoacrylate (Sellys, NZ). To provide additional strength and facilitate implantation, each electrode was threaded through a 3mm long section of a 26 gauge stainless steel cannula (PlasticsOne), so that approximately 10mm of the electrode protruded from the base of the cannula. The cannula was then glued in place with additional cyanoacrylate and allowed to dry. Once dry the electrode array was cut to approximate lengths, 4 mm for prefrontal and hippocampal electrodes, 6 mm for ATN electrodes. A gold Milmax pin was then secured to one end of the cannula to aid implantation. The four wires at the other untwisted end of the electrode were then separated out, the coating was removed with a naked flame and each wire was soldered separately to one pin on a block of Milmax connector containing four male/female pins (m/f; i.e. pin and socket). This allowed each electrode to be implanted independently. The soldered connections were then strengthened by coating them with epoxy resin and left for 24 hours to set. Once set, each electrode was tested for patency and to ensure there were no shorts between channels and then placed in a clean sealed box until implanted (figure 8.1). Ground electrodes were constructed from 12 cm lengths of 200µm silver coated copper wire (Jaycar, NZ). The ground wire was soldered on to a block of two m/f Milmax pins, bridging the two pins to create a common reference and ground channel.

Figure 8.1: An example of a completed recording electrode. A milimax connector containing four male to female pins (A) was soldered to the untwisted ends of four platinum iridium wires (B). The twisted wires (E) were passed through and secured to a 3mm length of cannula (D), which had a gold male-male milmax pin attached to aid implantation (C).

### 8.2.7. Electrode implantation surgery

Rats were implanted with the recording electrodes six to eight weeks after lesion surgery. All vapour anaesthesia and stereotaxic equipment was cleaned and disinfected with sterile medi-wipes before and after use. A subcutaneous injection of Carprofen (5mg/kg) was administered 1 hour prior to this second surgery for pain relief. Hartmans solution (sodium lactate, IP) was given for hydration. General anaesthesia was achieved by placing the rat in a small induction chamber with 4% isoflurane mixed with oxygen at a flow rate of 1500ml/min. Before mounting in the Kopf stereotaxic holder the rat's head was shaved and cleaned to remove loose fur. Then tail pinch and plantar reflexes were tested to ensure an appropriate surgical plane of anaesthesia. The rat was then transferred to the nose cone and mounted in the stereotaxic apparatus. Methopt Forte eye drops were applied with the addition of a moist gauze placed above and clear of the eyes. The site of incision was cleaned with sterilized gauze soaked in 4% chlorhexidine gluconate and the rat was given local analgesia to the scalp (0.2ml of 2mg/ml of Mepivacaine). The rat's body was kept warm during surgery with bubblewrap. Once a stable level of anesthesia had been achieved, the isoflurane level and oxygen flow rate were reduced to a maintenance dose of 2% and 1000 ml/min respectively. Waste gases were actively scavenged into the outside atmosphere via an exhaust system. If the animal appeared responsive to tail pinch or plantar reflex the isoflurane rate was increased to 4% until the rat was again unresponsive. Following incision, remaining membranes were removed with sterile cotton tips and the skull left to dry. Bregma and lambda were located using a stereotaxic-mounted drill and a dorsoventral coordinate was obtained at each of these sites to ensure the rat's head was flat. Six holes were then drilled around the inside perimeter of the incision and five anchoring screws inserted. The sixth screw was placed above the cerebellum to act as the primary ground/reference channel. The uninsulated ground wire was wrapped first around the ground screw and then around all remaining screws. Small craniotomies were then made above the sites of the PFC, HPC and AV recording electrodes (see table 8.1 for coordinates and figure 8.2). Each electrode was secured with a small amount of dental acrylic and allowed to dry before being removed from the mounting arm. The prefrontal electrode was always inserted first followed by the bilateral AV electrodes and finally the bilateral hippocampal electrodes. Once all the electrodes were secured in place a thin layer of dental acrylic was applied over the entire area. Once dry the Millmax pins used to insert the electrodes were removed. A dummy Millmax connector head cap was used to line up the four-pin connectors attached to the electrodes and the two-pin

connectors attached to the reference/ground electrodes. Dental acrylic was then used to cover all exposed electrode wires, the anchoring and reference screws and shaped into a headcap (figure 8.3). The incision around the head cap was cleaned with sterile saline and sutures used if necessary. Emla analgesic cream was applied to the scalp area following suturing and the rat was given additional Hartman's solution (1.0ml ip). At the completion of the procedure, the isoflurane was switched off and the animal was provided with 2 minutes of pure oxygen through the nose cone or in the induction chamber before being transferred back to a clean cage for recovery. Post-operatively, especially during the first week, both the researcher and laboratory technicians monitored recovery to check that the rat's wound was healing (no discharge, inflammation or open stitches), the rat was drinking (water bottle weighed daily), eating (food reduced, feces and rat weighed every second day) and returned quickly to being bright, alert and responsive.

**Table 8.1: Electrode coordinates**

PFC			
Distance B to L	AP coordinate	Lateral coordinate	DV (from dura)
≥0.64	+0.300	± 0.06	-.35
0.65-0.68	+0.305		
0.69-0.72	+0.310		
< 0.72	+0.315		
AV			
Distance B to L	AP coordinate	Lateral coordinate	DV (from dura)
≥0.64	-0.131	± 0.145	-0.488
0.65-0.68	-0.136		
0.69-0.72	-0.141		
< 0.72	-0.146		
HPC			
Distance B to L	AP coordinate	Lateral coordinate	DV (from dura)
≥0.64	-0.285	± 0.21	-0.28
0.65-0.68	-0.290		
0.69-0.72	-0.295		
< 0.72	-0.300		

B=bregma, L= lambda, AP = anterior- posterior, DV = dorsal-ventral



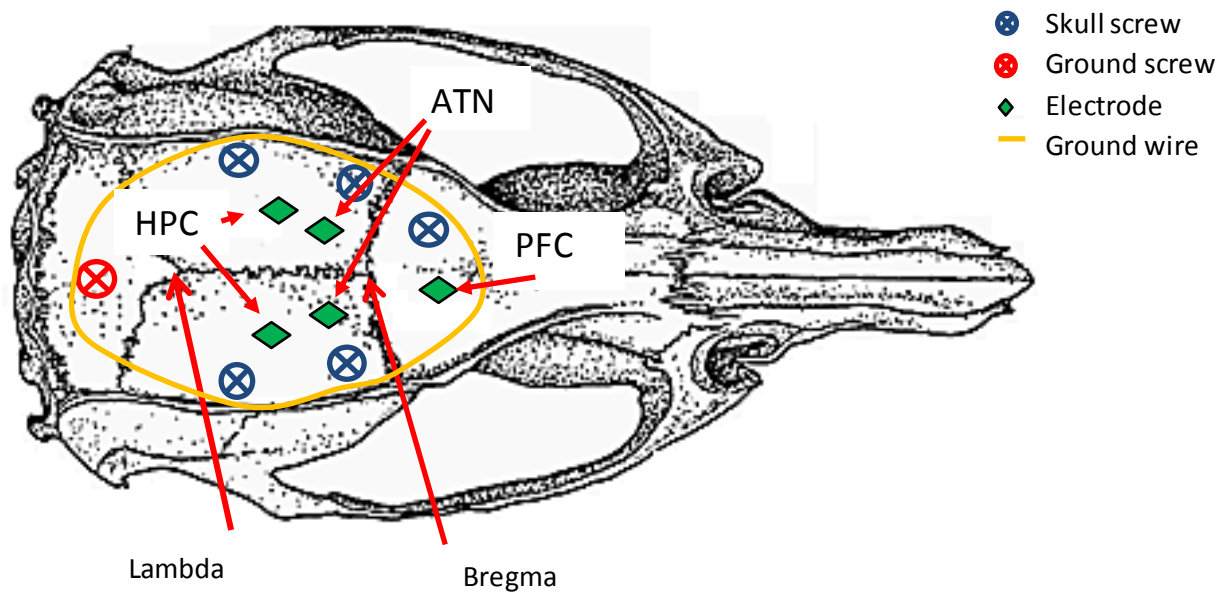


Figure 8.2: diagrammatic representation of a rat skull (adapted from Paxino's and Watson 1998) depicting the approximate location of the skull screws, ground screw, tetrodes and ground wire.

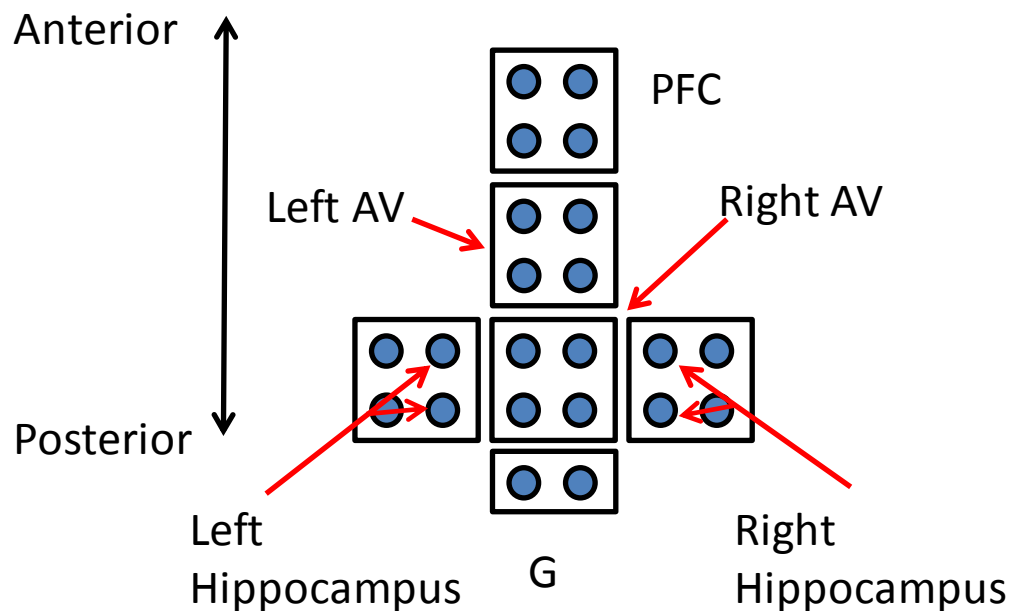


Figure 8.3: Diagram indicating the topographical layout of the milmax plugs on the rats head. G = ground electrode

### 8.2.8. Electrophysiological recording

Electrophysiological data was recorded with the OmniPlexD Neural data acquisition system (Plexon, Tx). The implanted recording electrodes were connected to the system via a custom made Milmax/Omnetics adaptor consisting of a bank of Milmax pins in the same configuration as the rats headcap, then soldered onto an 16ch electrode interface board (Neuralynx, Mo.) which connected directly to the Plexon digital headstage via an omnetics connector (figure 8.4). Single electrode signals were referenced to ground, filtered between 1 and 7500 Hz, multiplexed and digitized by the headstage and up sampled to 40KHz by the digital head stage processor (DHP). Digital signals were transmitted over a data link cable to an acquisition card connected to a computer running Omniplex server and Plexcontrol software (figures 8.5 and 8.6). The OmniPlex server split the wideband signal into two separate data streams. One stream was used for spike band activity, bandpass filtered between 300-6000Hz and sampled at 40kHz; the other stream containing field potentials and was bandpass filtered between 0 and 500Hz and sampled at 1kHz. All recorded channels were saved to the computer running the OmniPlex software for offline analysis.

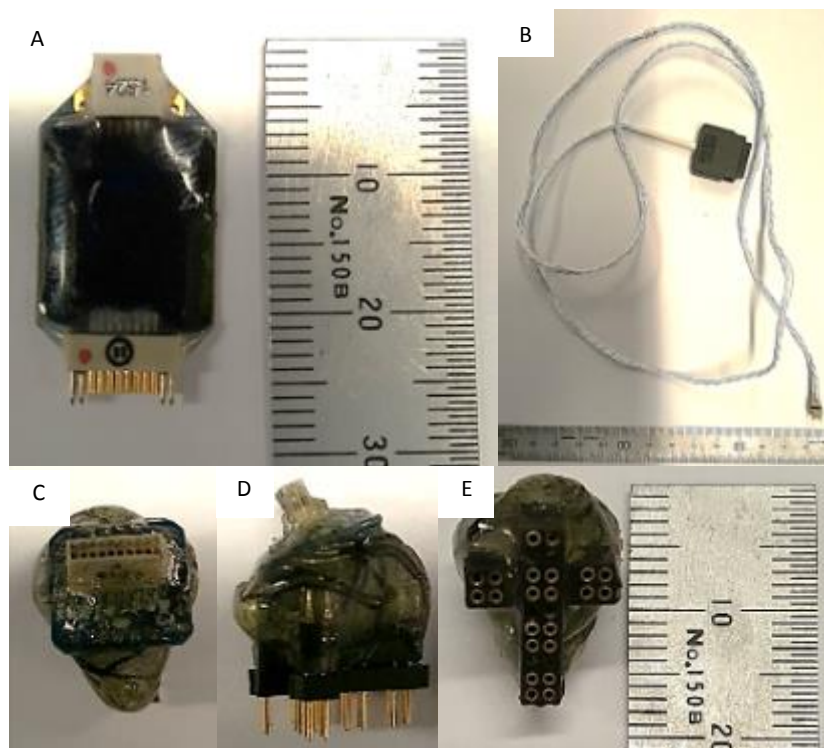


Figure 8.4: (A) Plexon digital headstage with inbuilt amplifier (B) headstage cable which connected the headstage to the commutator or DHP unit. C,D,E various views of the custom made omnetics to milmax adaptor used to connect the head cap on the rat to the recording headstage.

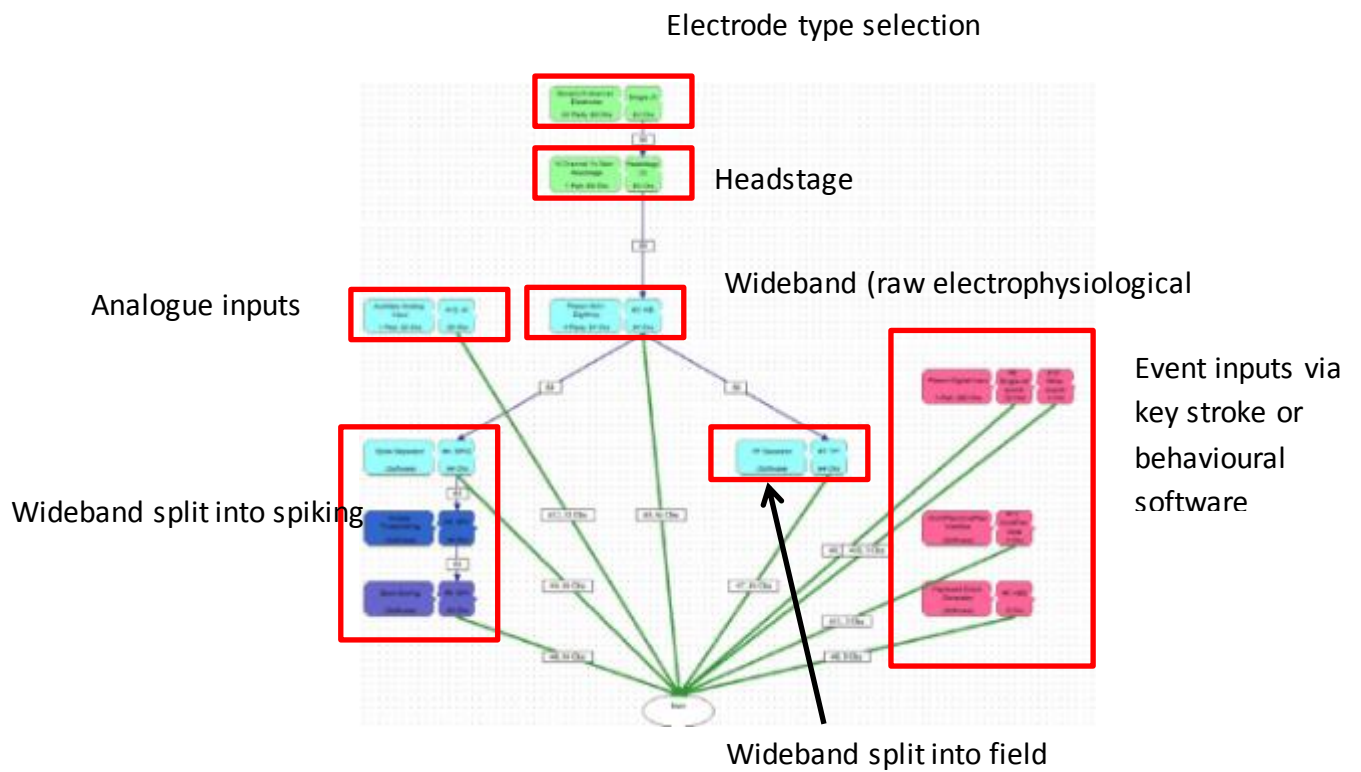


Figure 8.5: Screenshot of Plexservers typology tree for controlling the various inputs and outputs of the data stream

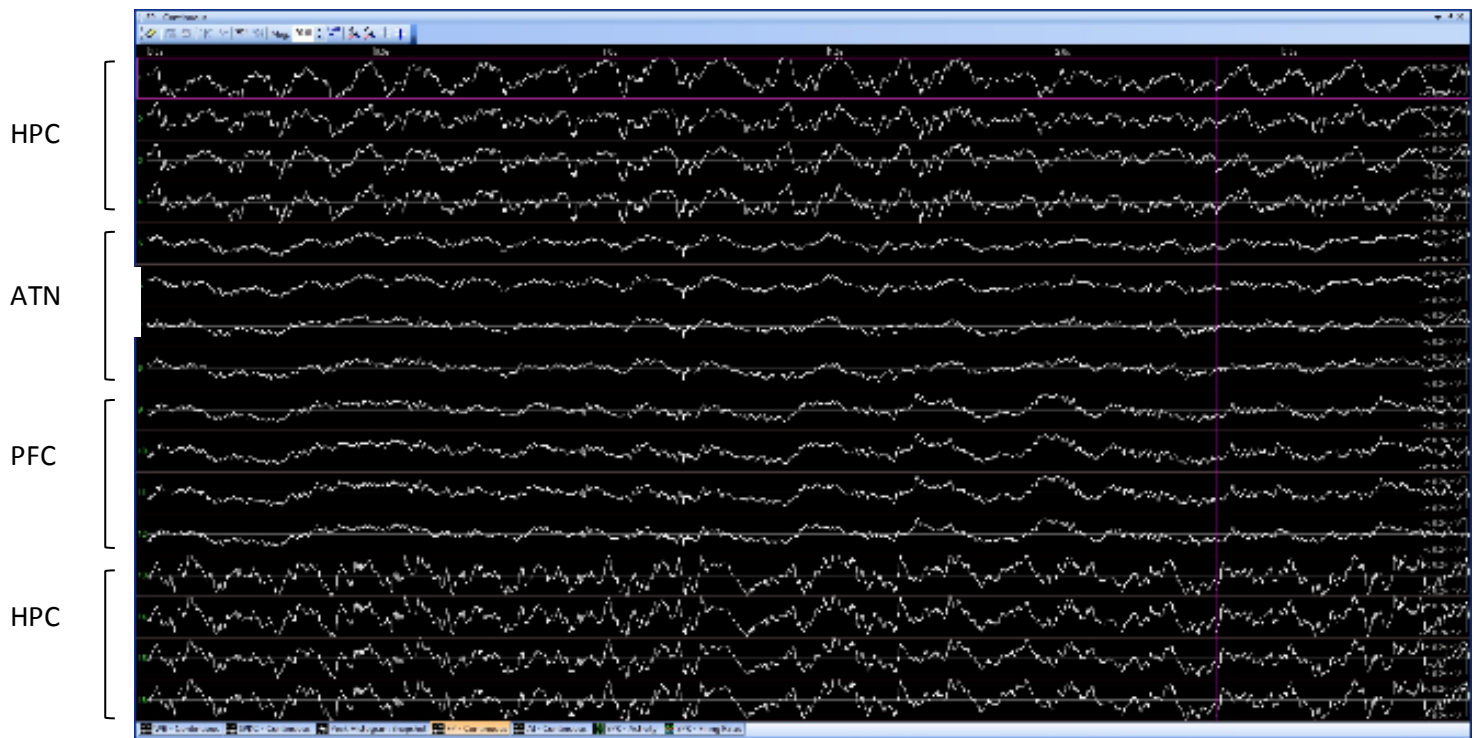


Figure 8.6: Screenshot from the Plexcontrol software depicting LFP recordings from the HPC, ATN and PFC in an awake behaving rat as a function of time.

500ms

### 8.2.9. Intraoperative baseline and tail-pinch recordings

At the conclusion of the electrode implantation surgery, once the head cap appeared well-set, electrophysiology recordings were taken from the ATN, HPC and PFC electrodes in each rat under isoflurane anesthesia. Rats were plugged into the headstage and the electrophysiology software was initiated. Once LFP activity had stabilized (approximately 5-10 seconds) a baseline recording was made for 1 minute under 2% isoflurane with an O<sub>2</sub> flow rate of 1 litre per minute. Next, a tail pinch recording was made. To ensure consistency across rats a weak peg was placed approximately 4 cm from the tip of the tail, and a 1 minute recording made. Tail pinch is a common procedure for driving rhythmic activity in the brain.

Following the electrode surgery the rats received 7 -10 days of recovery of single housing to avoid damage to the head caps. Subsequent to recovery rats were placed back on food restriction for the random foraging and RAM tasks. To mitigate the stress of social isolation the rats received at least two hours a day of monitored social housing (figure 8.7). This consisted of placing mixed groups (both MTT and sham rats) of 6 to 8 rats in a large cage with two pieces of PVC pipe a chewing block and some nesting paper. Following social housing each day the rats were returned to their individual cages for feeding and stayed there during their light cycle (8pm-8am).

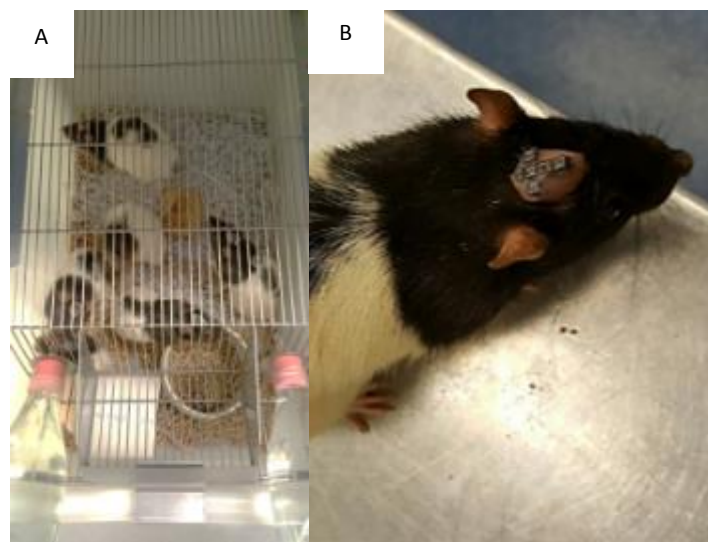


Figure 8.7: An example of a social housing cage (A), in which rats were allowed to interact for at least 2 hours a day and while being tested on the behavioural tasks. (B) An example of a finished head cap on a rat

### 8.2.10. Random foraging in an open field

Approximately 3 weeks after electrode implantation rats were given a random foraging task. This task served as a control task for local field potential recordings in the radial arm maze as it provided exploration of a spatial context without any task specific demands. This task was conducted in a large square arena, 80 cm x 80 cm x 50 cm high with grey walls and a white plastic sheet on the bottom to facilitate tracking the rat's movements (figure 8.8). Prior to the start of each trial approximately 15g of chocolate drops were sprinkled randomly across the floor of the box. Each rat received one three minute trial daily for five consecutive days. Each day the rat was removed from its social housing cage and its dust cap removed. It was then plugged into the electrophysiology system via a 16 channel headstage, which was connected to a tether to a digital headstage processor via a commutator. After the rat was plugged in and placed in a separate cage, the electrophysiology recording software was initiated (Plexcontrol) and once recording had stabilised (typically 5-10 seconds) the rat was removed from the separate cage and placed in the arena. The rat's movements were recorded using Ethovision XT 5.1.



Figure 8.8: Example of a rat performing the random foraging task (A) and overhead commutator and video camera setup (B).

### 8.2.11 Rehabilitation to the RAM

Two days after the random foraging task the rats were rehabilitated to the RAM over two days. On day 1 cage groups of 3-4 rats were placed on the maze for 10 minutes with

chocolate drops sprinkled down the arms and piled up in the food wells, which also housed inaccessible chocolate drops to control for odour cues. On the second day rats were habituated singly for 3 minutes each. Approximately 8-10 chocolate pellets were placed in each well and the doors were lowered and raised repeatedly.

#### 8.2.12. RAM testing with simultaneous electrophysiology recording

Formal training in the RAM began the day after re-habitation. Each food well was now baited with 2 chocolate drops before the trial commenced. At the start of a trial the rat was plugged into the headstage and then placed in the centre of the RAM with all doors raised to block access to the arms (figure 8.9). The electrophysiological software was initiated and once recordings had become stable the trial began. To pair electrophysiology with behaviour manual time-stamps were added to the data file using three keystrokes. One was pressed as soon as the rat entered a correct arm (base of tail), another that the rat had entered an incorrect arm, and the third to indicate that the rat had returned (base of tail) to the central hub. Because the keystrokes relied on the experimenter's reaction time a compensatory 500ms epoch of LFP data was added either side of the timestamp. Electrophysiological recordings were stopped at the end of each trial and the rat unplugged from the headstage and returned to its social cage.





Figure 8.9: A rat performing the radial arm maze task with simultaneous electrophysiological recording.

### 8.2.13. Mid-trial delay RAM

Following the standard task, rats were tested for 6 days on a mid-trial delay task in the RAM. In this task the first four arm choices were run in the same manner as the standard task, but once the rat returned to the centre after the fourth correct arm entry it was held in the centre hub for a 5 minute delay. Almost invariably these first four choices were all correct in all rats. This slight procedural variation placed additional demand on working memory resources to increase task difficulty. During the 5 minute delay the rat was carefully removed from the maze and a clear Perspex cylinder was inserted in the central hub to prevent the rat from chewing or climbing the doors. To prevent the rat from escaping a square sheet of clear Perspex was placed on top of the cylinder with a hole in the centre large enough for the headstage tether to fit through. Following the five minute delay the Perspex cylinder and sheet were removed and the rat was allowed to locate the final four arms using the usual procedures. For this task three separate electrophysiology recordings were made each day. One file contained recordings from the first four arm choices (low cognitive load), another contained recordings from the 5 minute delay (no specific task demands) and the last file contained data from the remaining arm choices (high cognitive load); each of these epochs was analysed separately. As for the standard task keystrokes were used to indicate the

selection of a correct arm, an incorrect arm or return to the centre of the maze. Again an additional 500ms buffer was added either side of the timestamp to account for experimenter reaction time.

#### 8.2.14. Standard RAM with beam breaks

Following the delay RAM task, rats were given a three week break before further testing. At this point, 3mm diameter infrared (IR) beam breaks, consisting of a transmitter opposite a receiver (Adafruit Industries) were fixed 3 cm above the arm floor at two locations down each arm. One pair of sensors was located 15 cm down the arm, to ensure the rat had entered the arm, and the other pair 12 cm before the end of the arm, that is, just before reaching the food well, giving a 38 cm distance between IR beam pairs within the arm (see figure 8.10). The beam breaks were powered by two separate 5 volt DC power outputs coming from analogue I/O ports on the Plexon chassis, each supplying power to four arms (figure 8.10). To read a digital signal when the IR beam was broken a 10K resistor was soldered between the signal and power wires of the IR beam receiver. The signal wires from each of the 16 IR receivers were fed into the digital input card on the Plexon chassis and automatically timestamped on the Plexon file with 25 microsecond resolution when the IR beam was interrupted by a rat.



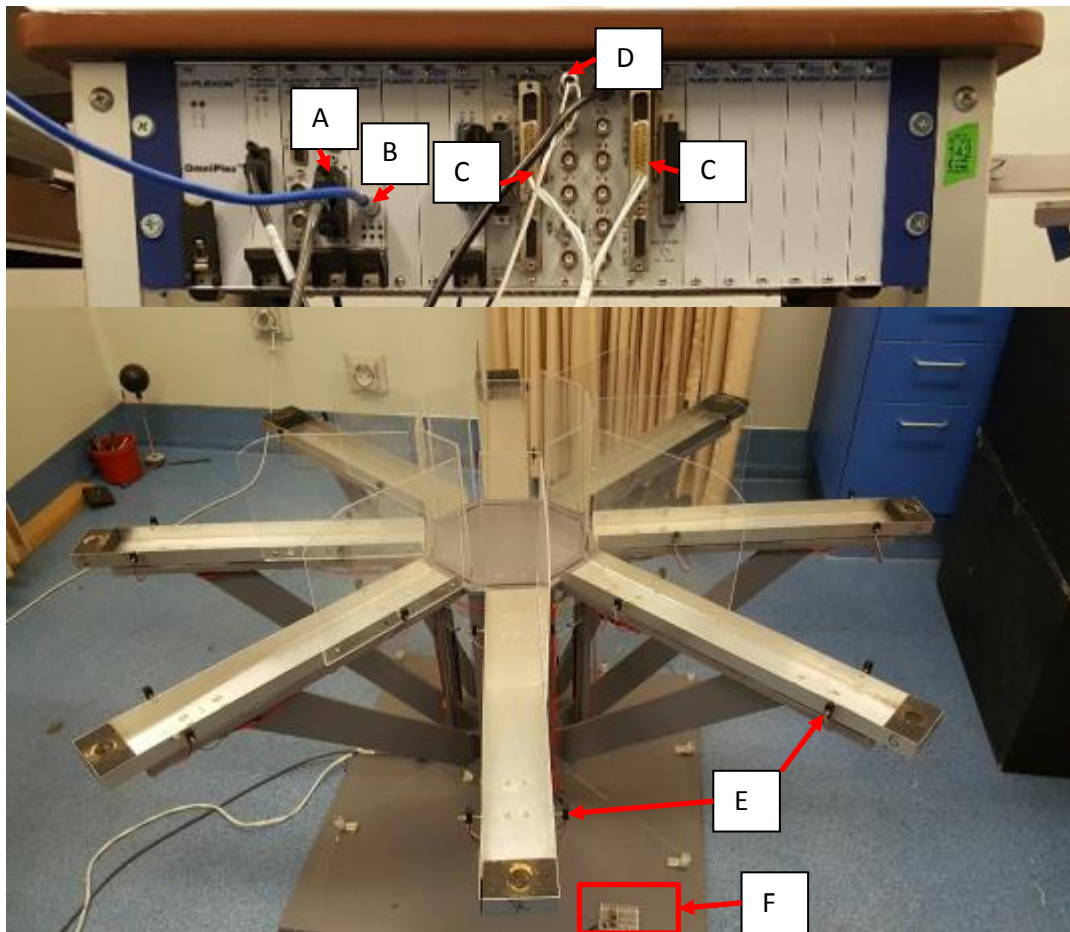


Figure 8.10: A) 16 channel digital input from the IR receivers; B) Datalink cable connecting the chassis to the DHP unit; C) 5 volt DC power supplies for the IR beambreak; D) Input line sending a signal from a Pulsepal to synchronise the video recording and electrophysiology; E) IR beam breaks fixed to the arms of the maze; F) LEDs to synchronise video and electrophysiology.

### 8.2.15. Standard RAM with beam breaks: Electrophysiology data collection

Electrophysiological recordings were only taken from two, 2-day blocks (4 days total). For the first six days of testing, rats were run on the standard working memory task (no delay) without the headstage plugged in. Recordings were made on days 7 and 8 and then days 11 and 12; days 9 and 10 were run in the same manner as the first six days. On electrophysiology days a video camera fixed next to the commutator above the central hub of the maze recorded the rat's movements. Videos were recorded on a separate computer and initiated immediately after plugging the rat into the headstage before anything else. Next the electrophysiology software was started and electrical recording was initiated once field

potentials were stable. In order to synchronise the neural recordings and video an open source pulse train generator (PulsePal V1.0; Sanders & Kepecs, 2014) with a split output cable was used. One end of the cable connected to an analogue channel on the chassis, which was sampled at 1k Hz and the other end connected to a bank of LED's fixed to the base of the maze within the camera frame. Before the doors were dropped and the trial started a 1second monophasic (+ve) 5 volt pulse was sent from the PulsePal. This sudden jump in voltage was recorded on the analogue channel and simultaneously illuminated the bank of LED's, which were recorded on the camera. Rats were then allowed to run the task in the same fashion as before. To equate the LFP data recorded in this task with the previously recorded data using key presses the IR sensors closest to the central hub were used as a reference point. Consistent with the previous tasks the beam break was activated approximately when the rats back legs crossed the threshold of an arm and a 500ms epoch of data was collected either side of the initial beam break for analysis (figure 8.11).

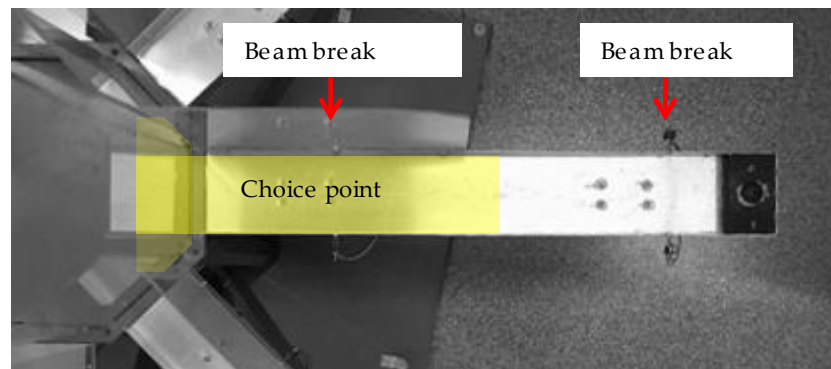


Figure 8.11: Location of infra-red beam breaks and choice point used during the later standard RAM testing.

#### 8.2.16. TBS stimulation in awake rats

A secondary aim of this experiment was to determine possible ATN electrical stimulation protocols that may improve spatial memory in rats with MTT lesions. Given that many of the cells in the anteroventral nucleus fire in theta bursts, a theta burst stimulation (TBS) protocol was selected (Tsanov, 2011a). Before applying this stimulation protocol to behaving rats, both tolerance and the impact of ATN TBS stimulation on the HPC and PFC was tested. For

this task a new custom made headstage that allowed ATN stimulation and HPC/PFC recording was used. Stimulation was provided by an open source pulse train generator connected to a stimulus isolator (model 2200, AM Systems). Previous research stimulating the ATN in behaving rats has used monophasic pulses and currents up to 300  $\mu$ amps in an attempt replicate the parameters used in clinical settings (Hamani et al., 2010). For this reason monophasic square pulses at 200 $\mu$ amps (4-6 volts) were selected. Rats were stimulated with bursts of four monophasic pulses (100 Hz; each 0.1 ms in duration) with an inter-burst interval of 130ms resulting in a bursting frequency of  $\sim$ 7.7Hz to more closely approximate theta in a behaving rat. Each rat was plugged into both the stimulation cable and the electrophysiology system and was placed in a standard cage. To accurately determine when each stimulation epoch began a two-way BNC connector was attached to the PulsePal. One BNC cable connected to the stimulus isolator and the other was fed into a single digital input channel on the Plexon chassis, so that every time a monophasic pulse was sent from the PulsePal it was time-stamped on the plexon file. Once each rat was plugged it was placed in a clean standard cage and Plexcontrol was initiated and for the first 30s only neural activity was recorded. After 30sec the PulsePal was manually initiated and a one second pulse train was given to the rat, then another 30 seconds of recording followed by one second of stimulation and so on. In total each rat received five 1 second stimulation trains over 3 mins. The 5secs of LFP data preceding the one second train of TBS stimulation was compared to the 5sec of LFP following stimulation.

### 8.2.17. TBS stimulation during the first 4 arm entries of the RAM

Next the TBS protocol described above was applied to behaving rats during the first four arm choices (or a maximum of 90secs) in the standard RAM task. To allow stimulation and recording while the rat was running in the RAM a light weight stimulation cable and head stage adaptor were fabricated (figure 8.12). The stimulation cable attach to the rat was then soldered onto a slip ring commutator (Adafruit) and the other end soldered to heavy gauge hookup wire which plugged into the stimulus isolator via banana plugs. To attenuate the current coming from the isolator a 30k potentiometer was soldered across the positive lead and then grounded. After the potentiometer the positive lead was split so the stimulation pulses could be constantly monitored. One end of the positive lead was soldered to a BNC cable plugged into an oscilloscope and the other end fed into the commutator.

The first four arm choices were selected in an attempt to facilitate encoding as previous research suggests that this period may be especially sensitive to MTT lesions (Dillingham et al., 2015). For this task, the rats were first given six days of standard RAM testing in the same manner as before except this time all trials were ended once a rat had made 10 arm choices. Electrophysiological recordings were made in the same fashion as the beam-break procedure. For TBS stimulation the rats were matched on previous performance within their respective lesion groups and then allocated to one of two groups. The first group received stimulation+recording on day one and group two recording only and then vice versa for groups on day 2. Stimulation+recording and recording only sessions were alternated across days and between groups so that stimulation was only applied once to each rat within a 48 hour period. Each rat received five alternating days of each condition (10 days total). For stimulation rats were plugged into the stimulation head stage (figure 8.12h, i) and then placed gently in the central hub of the maze. Video recording was started first then neural recording was initiated, once recordings had stabilised a synchronisation pulse was sent from a PulsePal. Just prior to the doors being lowered TBS stimulation was initiated and then the trial was started. Stimulation was stopped once the rat had returned to the central hub after its 4<sup>th</sup> arm choice (irrespective of any revisit) or once 90 seconds had elapsed. Due to time constraints only the behavioural data associated with this task were analysed

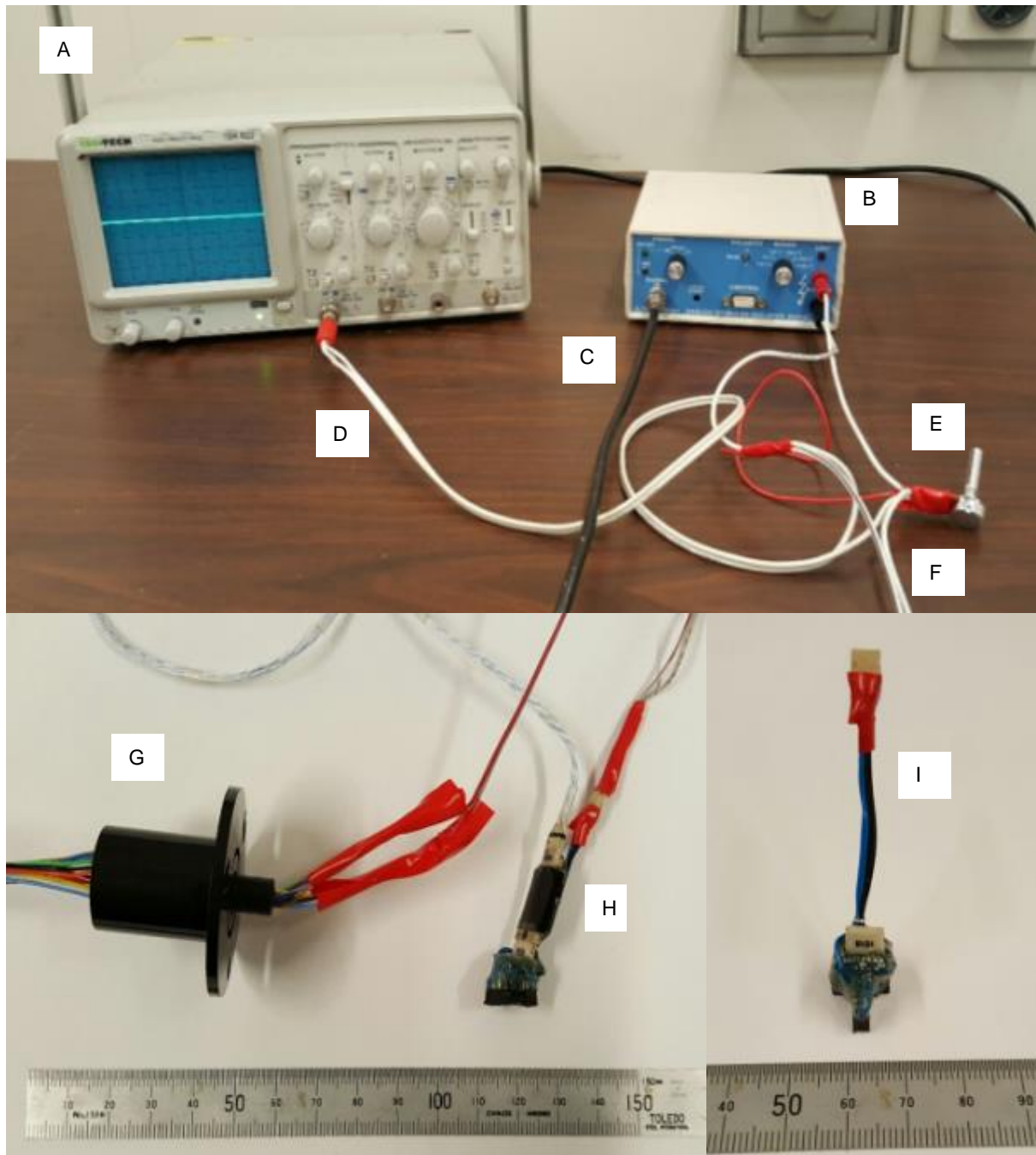


Figure 8.12: The setup of the stimulus isolator (B), oscilloscope (A), commutator (G) and the headstage (H) used during stimulation in the RAM. Pulse trains were sent via the PulsePal to the stimulus isolator (C). The current from the stimulus isolator was modulated with a 30k potentiometer (E) and the output was split so that one stream went to the oscilloscope for monitoring (D) and the other went to the commutator and on to the rat via a custom made adaptor (I).

### 8.2.18. Zif268 induction procedure

Examining the effects of ATN stimulation on other structures in the extended hippocampal system was the primary aim of this procedure, and the standard working memory task in the RAM was only used to examine the effect of 30 minutes of acute unilateral TBS in the ATN on spatial memory processing. Following a two-week break after the TBS stimulation in the RAM described above, the rats were returned to the RAM for a final five days of testing. The first four days were run in the same manner as previously, in that rats were only allowed to make a maximum of 10 arm choices, but at the end of each trial the rat received any rewards it did not manage to locate whilst in the maze, so that reward history did not influence zif268 induction. On the fifth day, the rats were placed in an operant box and connected to a stimulation cable for 30 minutes of unilateral ATN TBS stimulation using the same parameters as the previous task (see figure 8.13). At the end of the stimulation the rats were removed from the chamber and run in the radial arm maze. Following the maze procedure on the final day of testing each rat was placed alone in a cage in a dark quiet room for 90 minutes prior to perfusion.



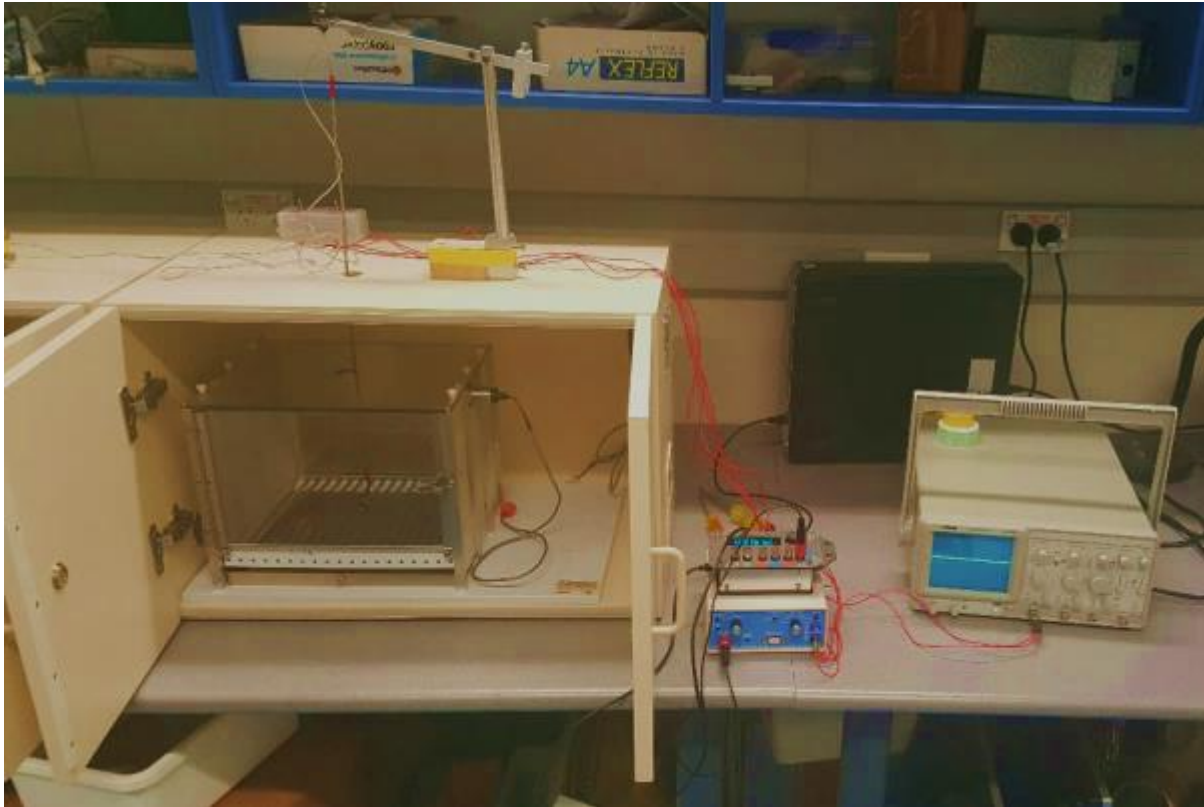


Figure 8.13: Layout of the stimulation boxes used for the 30mins of unilateral TBS to the ATN to drive zif268 induction.

### 8.2.19. Perfusion

Perfusions were performed in the same manner described in experiment 1 (see Chapter 6). At the completion of the perfusion the brains were allowed to post fix in additional PFA overnight at 4 °C before being transferred to a long term solution (20% glycerol in 0.1mol PB).

### 8.2.20. Histology

Brains were given at least 48 hours in long term solution before 40 $\mu$ m sections were taken in the coronal plane using a freezing microtome. In order to capture both electrode and lesion sites sections were collected in a series of four sections (one per vial) so that lesion verification and immunohistochemistry could be performed on approximately equivalent sections. Tissue was taken from the anterior portion of the prefrontal cortex (approx. +3.70 from bregma) back to the subicular formation (approx. -6.72 from bregma) and put into vials containing -20 °C long-term solution (40% 0.1mol PB, 30% glycerol and 30% ethylene glycol) for storage.

### 8.2.21. Lesion and electrode verification

Sections from one of the four vials from each rat were mounted on to subbed slides and allowed to dry overnight. Slides were then stained with Luxol blue, a myelin specific stain, and lightly counterstained with cresyl violet in the same manner described in experiment 1. In accordance with experiment 1 only those rats with at least 50% bilateral MTT lesions were included in the final lesion group. Lesions in this case were traced on atlas plates by JDA (Paxinos and Watson, 1998) and then the approximate area of remaining MTT fibres was estimated using in house software, although it should be noted that Luxol blues indicates the presence but not the viability of any remaining fibres. Like the MTT lesion analysis the approximate location of each electrode tip was independently determined by JDA and indicated on corresponding atlas plates.

### 8.2.22. Regions of interest for zif268

Zif268 expression was examined in the rostral aspect of the retrosplenial cortex (RSC) as well as the dorsal hippocampus. zif268 positive cell counts were made separately for the superficial and deep granular and dysgranular regions of the RSC, as well as CA1, the dentate gyrus and the combined CA3/4 region of the dorsal hippocampus. Images of each region were taken and analysed in the same manner used in chapter 6. To compare zif268 expression across groups, each ROI in the non-stimulated hemisphere in the sham group was normalised to have a mean of 100. The values for each rat were then expressed relative to the sham mean. Thus a mean of 100 in the MTT group would indicate no difference in zif268 expression relative to shams. A similar normalisation procedure was used to compare the stimulated vs non stimulated hemisphere, except that the mean of the TBS hemisphere was normalised to the non-stimulated hemisphere within each lesion group.

### 8.2.23. Data analysis local field potentials

Local field potentials from hippocampal, anterior thalamic and prefrontal electrodes were processed using Neuroexplorer version 5, matlab, SPSS and Microsoft excel. One patent channel per structure per rat was selected for all analyses of local field potentials (LFP's). Raw LFP data was first notch filtered at 50Hz with 1Hz notch width to remove line noise and then bandpass filtered between 1 and 110 Hz for recordings taken during surgery, and 2.5 and 100 Hz for recordings made during behavioural tasks. The band pass filter employed by neuroexplorer is similar to matlabs filtfilt function. The filtered LFP channels were then



visually inspected and any movement artefacts that appeared across channels were removed before spectral and coherence estimates were calculated. The frequency spectrum was broken down into the following frequency bands for analysis; delta (1-3.9 Hz), low theta (4- 8 Hz), high theta (8-12 Hz; i.e. alpha), beta (13-30 Hz), low gamma (30-48 Hz) and high gamma (52-100 Hz).

#### 8.2.24. Multitaper spectral estimation

Spectral analyses were performed separately for the ATN, HPC and PFC in each rat in Neuroexplorer using multitaper spectral estimation (time bandwidth product = 3; number of tapers = 5) with a 50% window overlap. This technique takes advantage of short time-window Fourier analysis to reduce artefacts caused by non-stationary elements in the data, as data can be assumed to be stationary within the short sliding time windows (Jones & Wilson 2005). For each spectral analysis each window was preprocessed by subtracting the mean before the tapers were applied. The data was smoothed with a Gaussian filter (3 bin filter width) and sent to Microsoft excel or matlab for further processing. To allow for effective comparisons across animals and recording sessions, data were normalised in matlab so the integral of the PSD over the frequency range was equal to one, by using matlabs 'mat2gray' function (Russel et al., 2006). This normalisation procedure accounted for variance in signal amplitude across recordings and animals as a result of factors such as electrode impedance and location. Critically, two measures were then obtained to provide maximum information about group differences within each frequency band: peak power and area under the curve. Peak power was calculated by taking the maximum normalised power for each region within each band per rat, thus giving a point estimate within each band. Area under the curve provided an estimate of total power within each band for each rat and was calculated by breaking the normalised data into smaller bands and using the matlab 'trapz' function, thus giving a global estimate of power within a bandwidth.

#### 8.2.25. Coherence analysis

Coherence is a functional measure based on the covariation between two EEG signals as a function of frequency. Coherence was estimated in Neuroexplorer using the same multitaper parameters described above. Coherence coefficients were calculated between the HPC-ATN, HPC-PFC and ATN-PFC separately between 1-110 Hz for recordings under anaesthesia and 2.5-100Hz in awake behaving rats. Only coherence coefficients between ipsilateral electrodes

were retained. Data were exported from Neuroexplorer into Microsoft excel. Again, Peak coherence and the area under the curve in each separate frequency band were calculated and compared across or within groups.

#### 8.2.26. Theta-phase Gamma-amplitude cross-frequency coupling

As discussed in chapter 5 theta-gamma cross-frequency coupling, especially within the hippocampus, is thought to play an important role in information storage and retrieval (Jensen & Colgin, 2007). Therefore an initial investigation into theta-phase gamma-amplitude coupling during correct arm choices in the beam break RAM task was performed. Because MTT lesions have been proposed to impair the encoding of spatial information the first four arm choices were examined (Dillingham et al., 2015). Coupling was calculated using the modulation index (MI; described by Tort et al. 2010). Raw LFP signal was bandpass filtered in Neuroexplorer for low (4-7.9Hz) and high (8-12 Hz) theta as well as low (30-48Hz) and high (52-100Hz) gamma. A Hilbert transform was then applied to the band-passed data to extract simultaneous theta phase and gamma amplitude in the hippocampus across correct choice epochs. The theta phase values and gamma amplitude values were exported in to matlab and an open source script from the “Attention-Circuits-Control Laboratory” run by Thilo Womelsdorf; available at <http://attentionlab.ca/doku.php?id=cross-frequency-coupling> was used to calculate a modulation index for the four interactions between low and high theta and low and high gamma for each rat.

## 8.3. Results

### 8.3.1. Histology MTT lesions

Sixteen of the 18 rats that received MTT lesion surgery met the criterion of at least 50% bilateral damage to the tract. Most of these successful lesions were complete or almost complete lesions to the tract (bilateral median, 97.5%; range, 70% – 100%). One excluded MTT lesion was due to minor bilateral damage (15%, 45 %) while the second exclusion had complete unilateral damage and only minor damage to the contralateral MTT (100%, 35 %). A further two of the 16 rats with satisfactory lesions were excluded because of surgical complications. One of these died shortly after electrode implantation and the other was found to have substantial damage to the prefrontal cortex during histological examination, leaving  $n = 14$  for analysis. However, three additional rats did not survive to the end of testing, as two rats dislodged their headcaps and were euthanized and the third died suddenly due to an unknown condition. The data from these three rats has been included in the initial tasks before any complications arose, which included the midtrial delay RAM task. A total of eleven rats with sufficient bilateral MTT lesions completed all behavioural testing. Two rats in the sham group were also removed from data analysis, both due to surgical complications. One of these sham rats had additional damage to the prefrontal cortex and another died shortly after electrode implantation surgery (final  $n = 10$ , which all completed all testing).

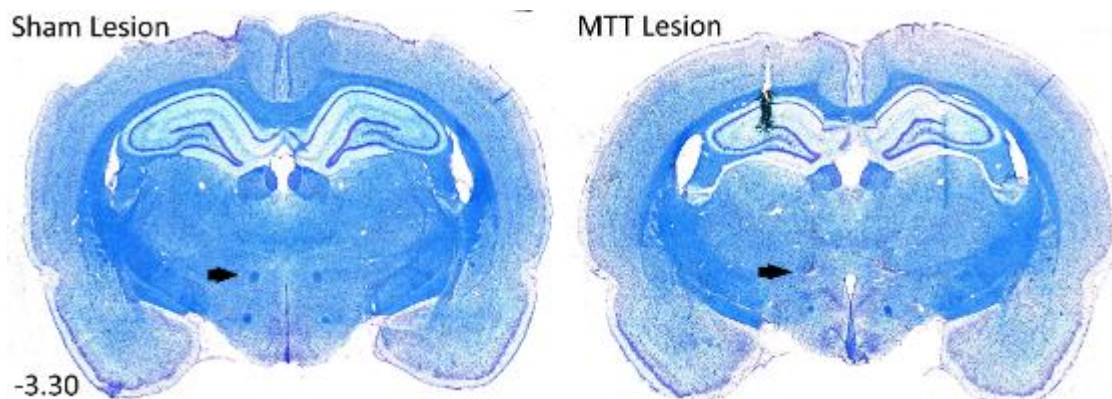


Figure 8.14: Photomicrographs of coronal sections stained with luxol blue and cresyl violet of an intact (Sham; left) and complete mammillothalamic tract lesion (MTT; right). Black arrows indicated the location or absence of the MTT. The hippocampal electrode tract is also visible in the left hemisphere (in the picture) of the MTT lesion section.

### 8.3.2. Electrode Placements: ATN

The anteroventral (AV) region / dorsal anteromedial region was the primary target for electrode placements because this region is part of a diencephalic- hippocampal theta circuit thought to propagate rhythmic oscillatory activity from the medial MB to the ATN and beyond (Jankowski et al. 2013). The anterodorsal nucleus (AD) is part of a lateral head direction circuit (Aggleton et al., 2010). Hence electrodes located in the AD or the most anterior aspect of the AV / bed nucleus of stria medullaris region (figure 8.15) were removed from data analysis. In total 22 of the 28 ATN electrodes in the included MTT rats were located within the target region (figure 8.15). Of the six discounted electrodes in this group, three were located in the most anterior AV and another three in the AD. In the sham group 16 of the 20 ATN electrodes were located within the target region. All four of these excluded cases were too anterior in the AV (figure 8.16). Because ATN and HPC electrodes were located in both hemispheres only data from one patent electrode in the same hemisphere per structure in any single rat were used for all analyses.

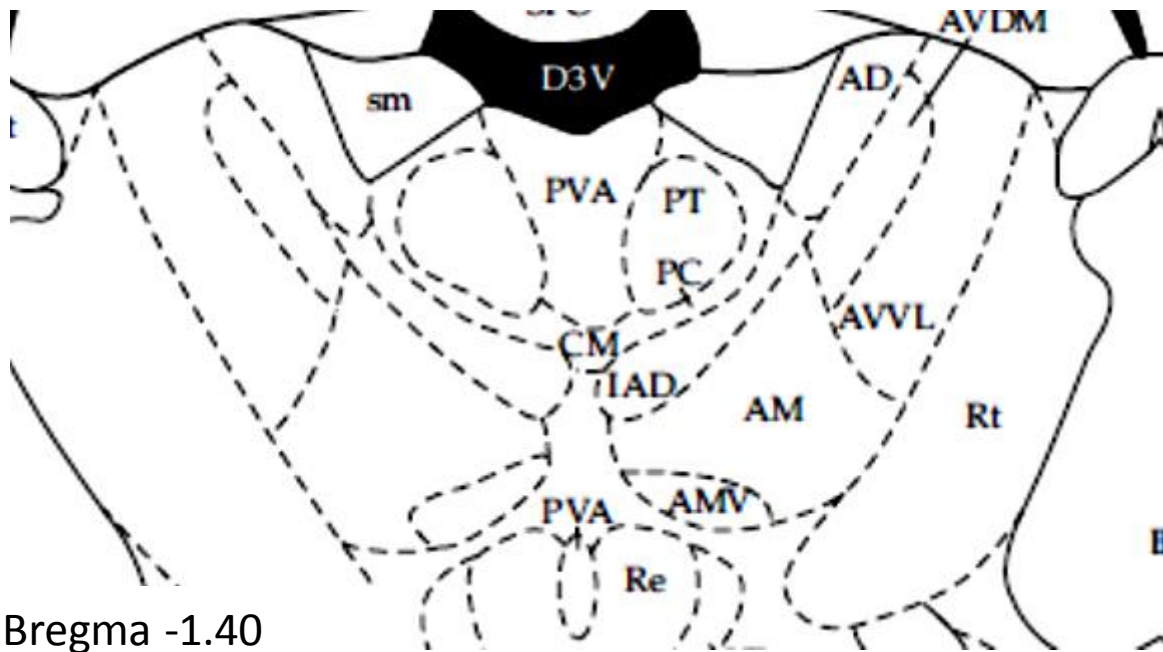
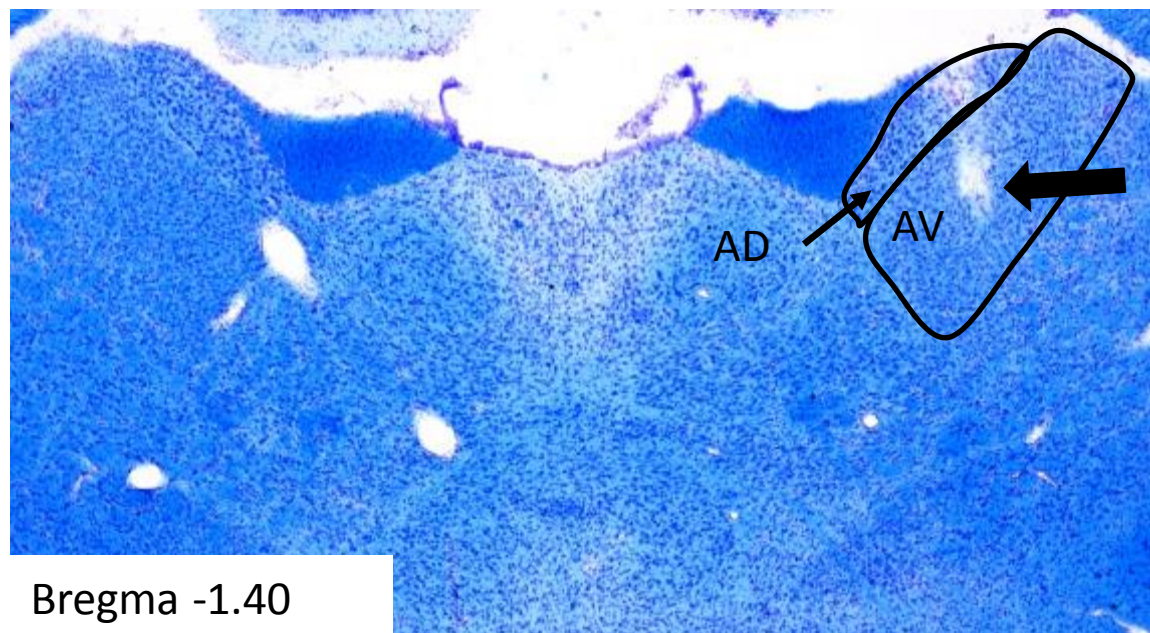


Figure 8.15: Photomicrograph of a coronal section at the level of the anterior thalamic nuclei stained with luxol blue and cresyl violet indicating the location of an electrode in one rat (top image; black arrow) and the corresponding atlas plate (Paxinos and Watson, 1998) indicating the thalamic nuclei.



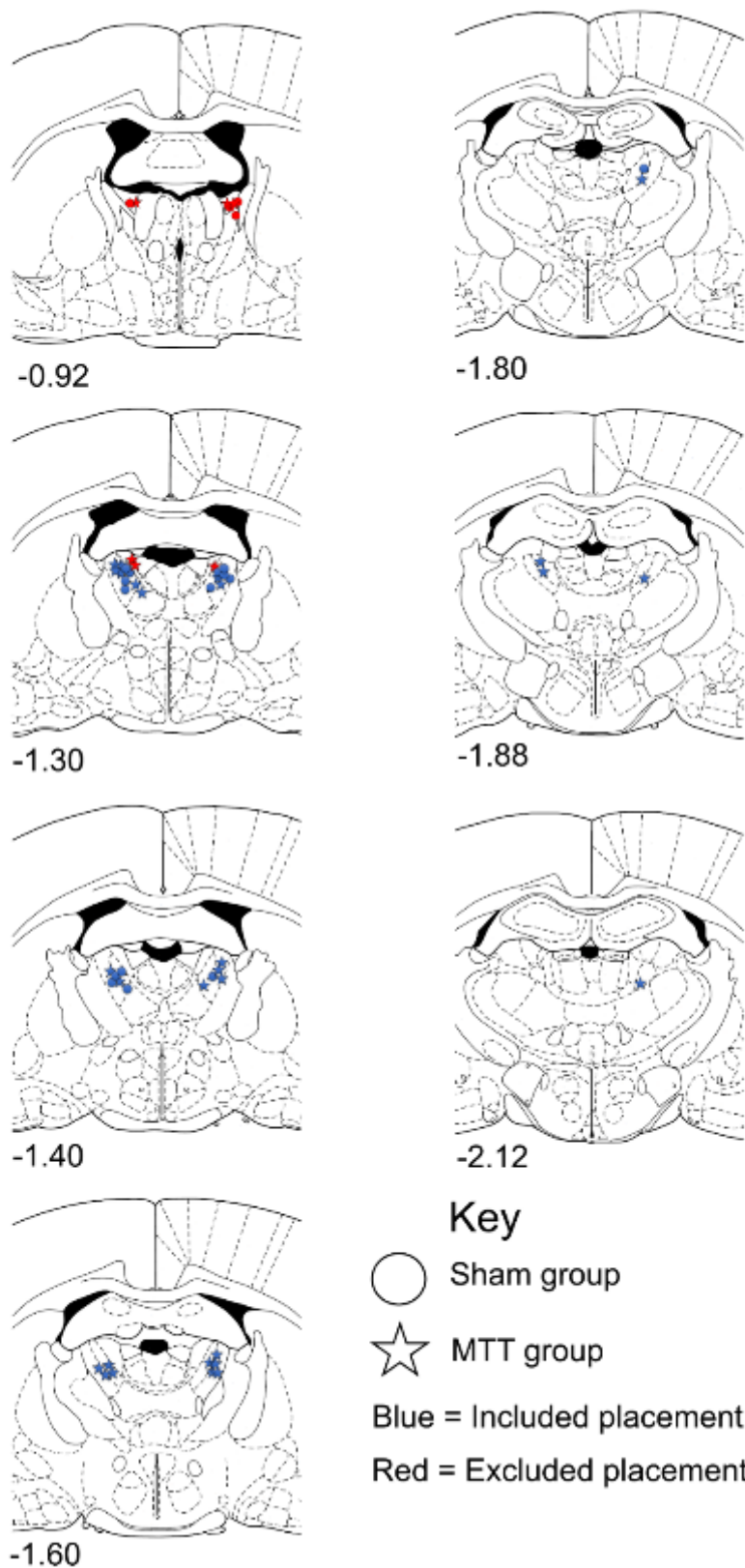


Figure 8.16:  
coronal atlas plates through the entire extent of the anterior thalamic nuclei indicating the approximate location of all ATN electrodes.

### 8.3.3. Electrode placements: dorsal hippocampus

Dorsal hippocampal electrodes were targeted at the region of the striatum lacunosum moleculare of the pyramidal CA1 cell layer and hippocampal fissure. This region was selected because of its robust oscillatory output, particularly in the theta frequency (Buzsaki, 2002; see figure 8.19). Because the oscillatory phase of hippocampal LFP differs across the dorsoventral aspects of the CA1 region, only those electrodes located below the pyramidal cell layer of the CA1 (that is, below number 6), but not at or below the granule cell layer of the dorsal blade of the dentate gyrus (that is, between numbers 6 and 15 inclusive in figure 8.19), were included for analysis (figure 8.18 shows an included case). In the MTT lesion group, one of the 28 dorsal hippocampal electrodes was located outside of the target region and was excluded from all subsequent analyses. In this case the electrode was located in the CA4 region (also known as the hilus of the dentate gyrus region). In the sham group four of the 20 dorsal hippocampal electrodes were not located within the target region and were excluded. One of these electrodes was located in the hilus of the DG, two in the ventral blade of the DG and another dorsal to the CA1 cell layer (figure 8.18). As per the ATN electrodes one patent hippocampal channel per rat was used for all analyses.

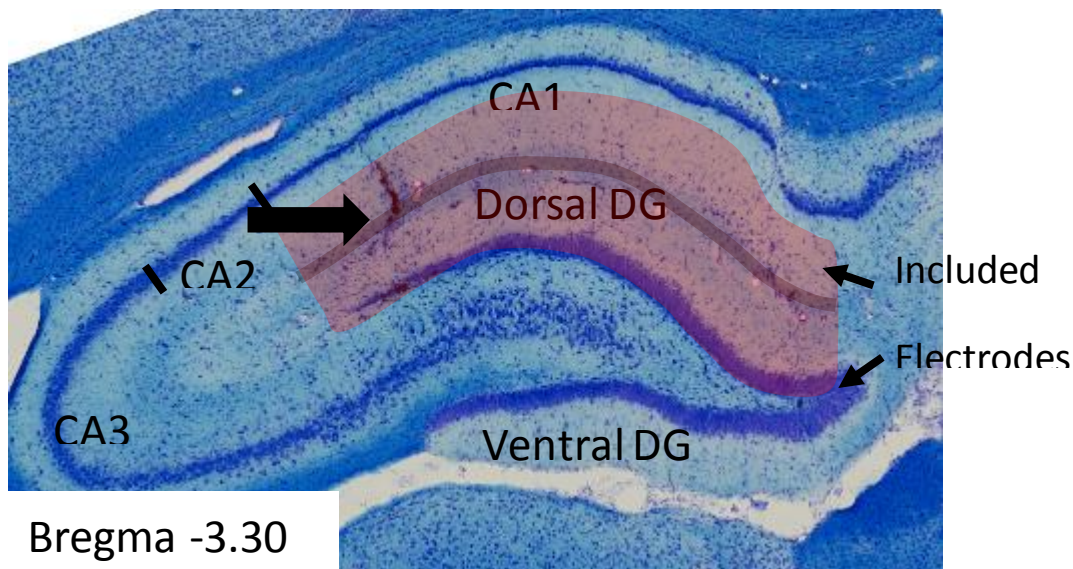


Figure 8.17: Photomicrograph of a dorsal hippocampal section stained with luxol blue and cresyl violet showing an acceptable electrode placement (black arrow). Hippocampal subregions are also indicated.

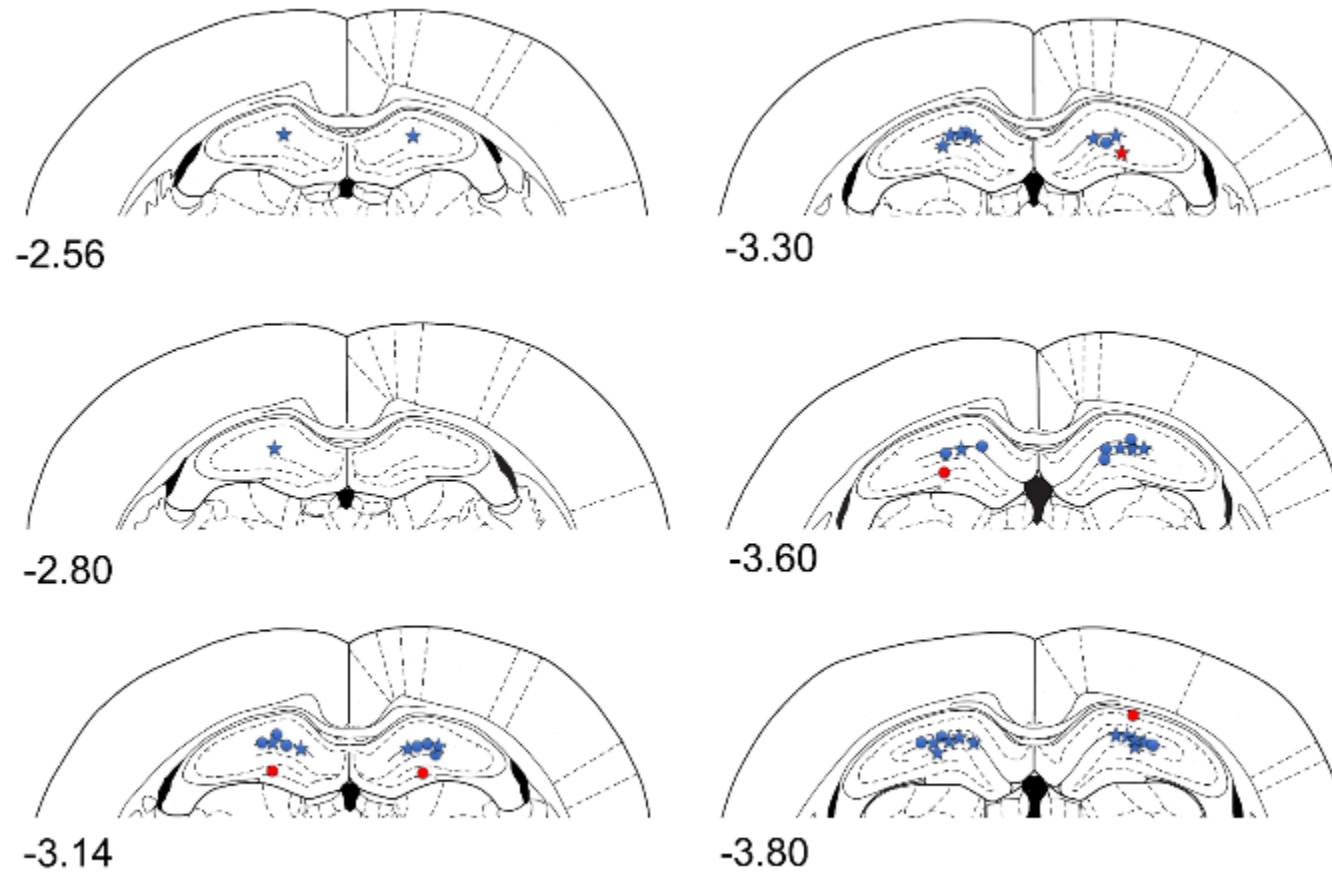


Figure 8.18: Coronal atlas plates indicating the approximate location of all dorsal hippocampal electrodes in both the sham and MTT group. Stars denote MTT rats and circles sham rats. Red indicates cases excluded from data analysis.



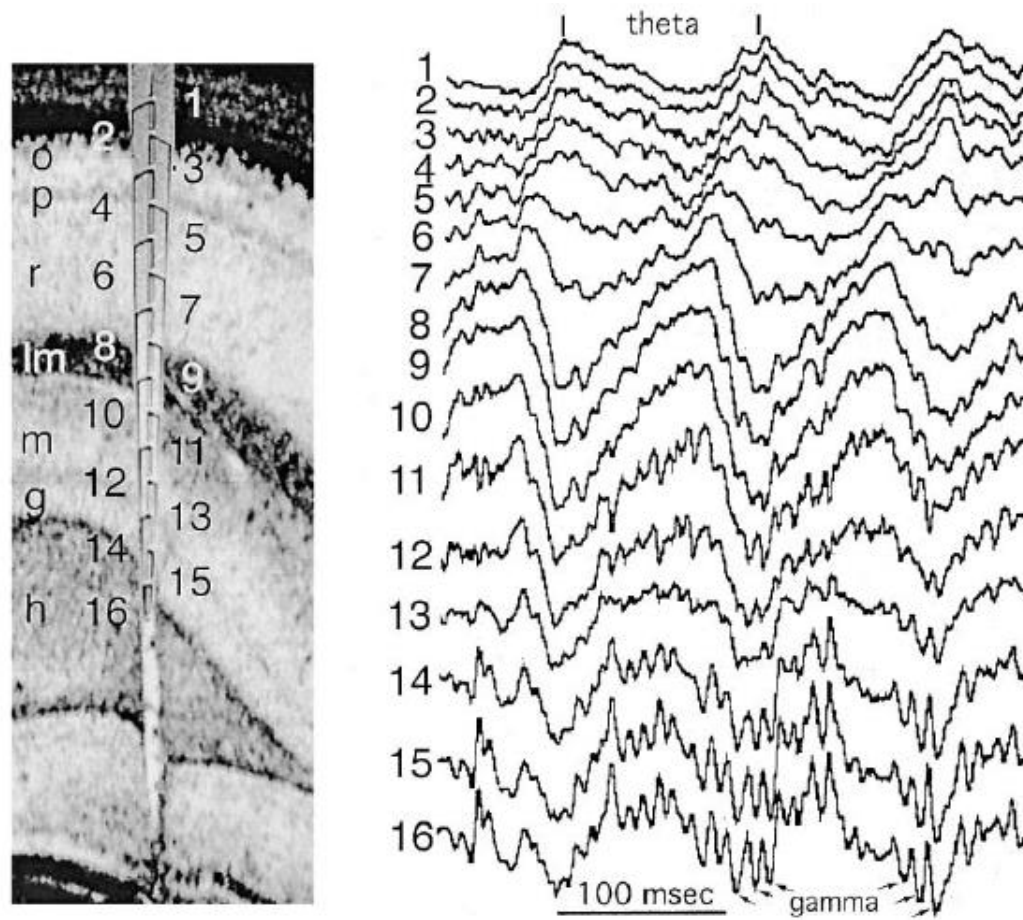


Figure 8.19: Adapted from Buszaki (2002) Silicon multi electrode array inserted into the dorsal hippocampus indicating differences in local field potentials across hippocampal subregions.

### 8.3.4. Electrode placements: mPFC

The prefrontal cortex of the rat is divided into three parts, the medial, orbital and lateral regions (Ongur & Price, 2000). For this study two regions within the medial subdivision were of primary interest, the infralimbic (IL) and prelimbic (PL). These two regions are most often associated with memory processes, and are connected to both the hippocampus and ATN (Hoover & Vertes, 2007; Jankowski et al., 2013). In all cases the mPFC electrodes were located within one of these two regions (figure 8.20). In the MTT group nine rats had electrodes located in the prelimbic cortex, with the electrodes of the other five MTT rats located in the infralimbic cortex. In the sham group seven rats had electrodes located in the prelimbic cortex and three rats had electrodes located in the infralimbic cortex (figure 8.21).

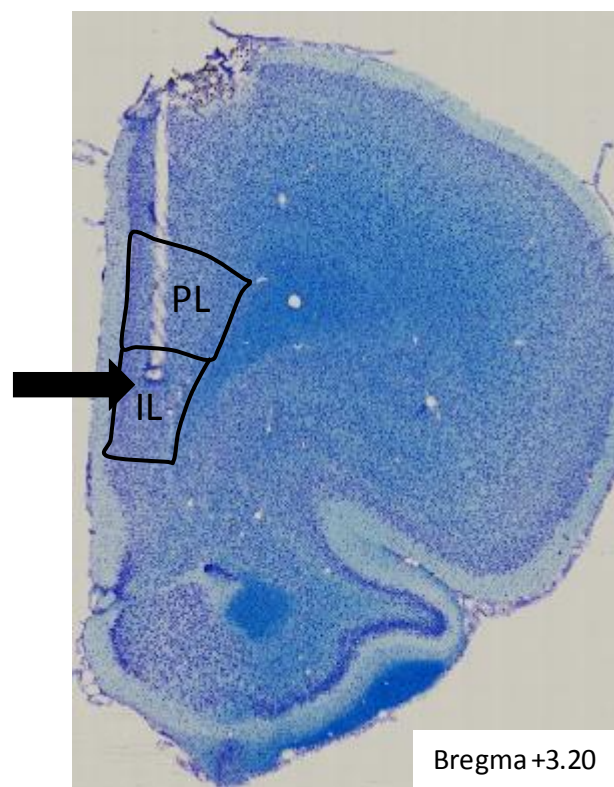


Figure 8.20: Photomicrograph of a prefrontal cortex section stained with luxol blue and cresyl violet showing an example of a recording electrode located within the infralimbic cortex (black arrow).

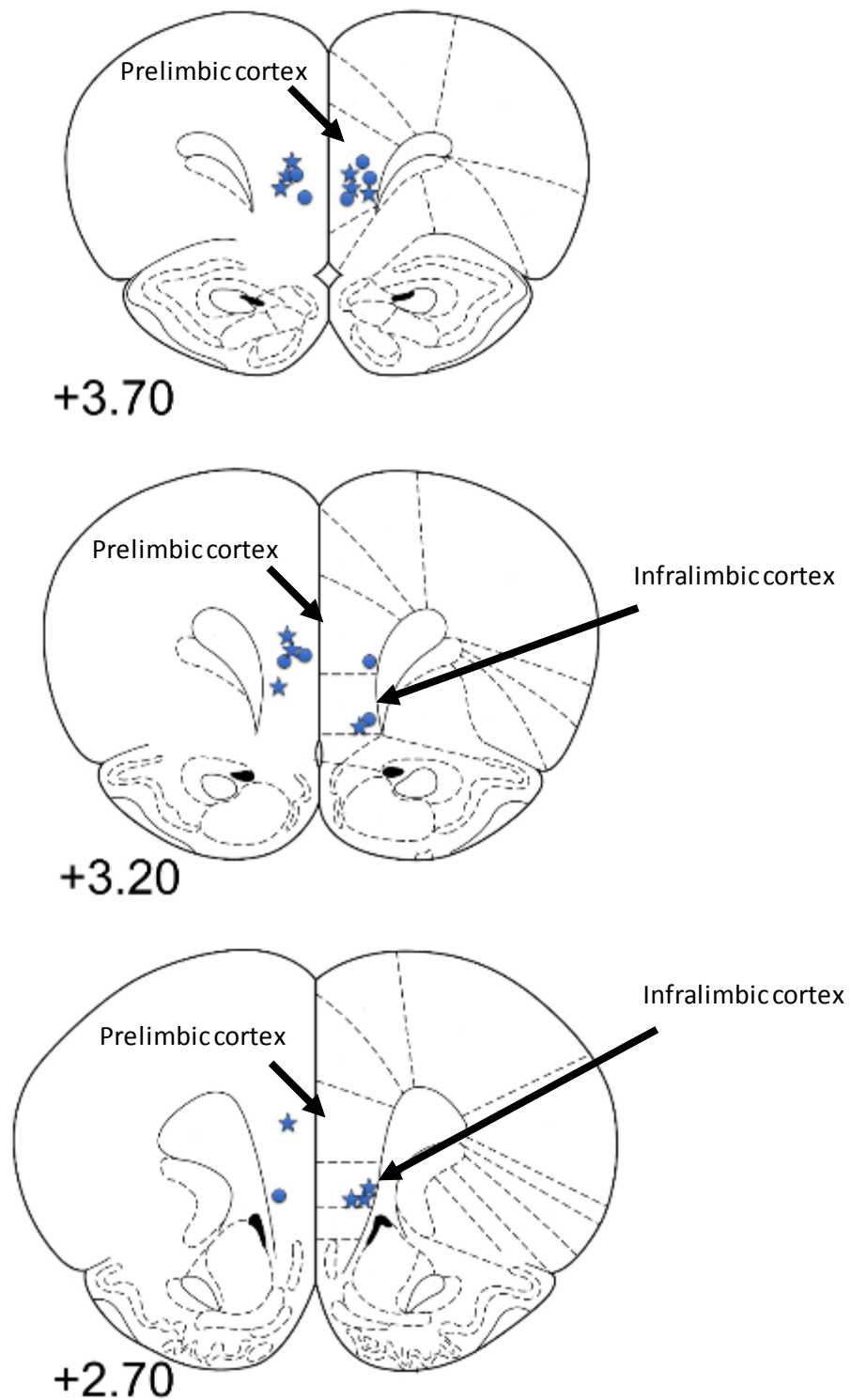


Figure 8.21: Coronal atlas plates indicating the approximate location of all prefrontal electrodes.

### 8.3.5. Data analysis

#### 8.3.6. Behavioural data

Repeated measures ANOVA and students t-tests were employed for all behavioural data and comparisons of immediate early gene expression in the retrosplenial cortex and hippocampus.

#### 8.3.7. Electrophysiological data

Two measures were used to examine differences in power and in coherence within each frequency band. Peak power or peak coherence described the maximum power or coherence value from each rat within in each band. In addition, a more global measure of power and coherence was obtained by calculating the area under the spectral power or coherence within all values for each frequency band (Area) for each rat. Previous evidence has suggested that theta and gamma frequency bands might be especially important (Nyhus & Curran, 2010; Colgin, 2011; 2013), so these bands were divided in to low and high component in the present study. Frequency bands were therefore defined as low theta (4-8 Hz), high theta (or alpha; 8-12 Hz), beta (13-30 Hz), low gamma (30-48 Hz) and high gamma (52-100 Hz). It was not known a priori which frequency would be sensitive to lesion effects in terms of power or coherence. Due to the large number of statistical tests required to examine each frequency band separately it was not feasible to control the family-wise Type I error using standard parametric statistics. There was also unequal variance and non-normally distributed data between the sham and MTT lesion groups. Therefore a Mann-Whitney U test was used to compare peak and Area ("total") values in each frequency band between the MTT and sham groups; a Wilcoxon rank sum test was used to analyse within-subject measures. Importantly, the family-wise Type I error was controlled by using an p value calculated for each test using a Monte Carlo estimation which permuted the data 10000 times and gave a 99% confidence interval (CI) around the p-value (Maris & Oostenveld, 2007; Maris et al., 2007). The critical alpha value was set at 0.05 for all statistical tests and deemed significant if the 99% CI did not fall below 0.05.

For each of the three RAM-LFP recording tasks a comparison of spatial memory errors between groups is presented first, followed by a comparison of power, then coherence during aggregated choice epochs. As outline in the method section, a choice epoch was defined as 500ms either side of a rats hind legs crossing the threshold of an arm (1 sec total for each). Where sufficient data was available correct and incorrect arm choices were

compared within a group to examine power and coherences changes associated with task outcome. Because the amount of recording differed for each task the total length of data analysed is stipulated separately for each task. For the first RAM-recording task, no distinction was made between early and later trials, whereas for the later mid-trial delay and t beambreak RAM tasks data during choice epochs for the relatively easy early arm selections (pre-delay and first four arm choices) and data from later arm selections (post delay or last four arm choices) with greater cognitive demands were both examined.

### 8.3.8. Post lesion testing in the radial arm maze

#### 8.8.9. Post-surgery spatial working memory in the radial arm maze (RAM) prior to electrode surgery

Rats were tested on the standard working memory version of the RAM following the first surgery to establish the lesion deficit. Rats with MTT lesions were profoundly impaired on this task diverging from sham performance by the third block of testing, with the Sham group acquiring the task quickly whereas the MTT group showed no evidence of improvement with training (Lesion,  $F(1,22) = 89.34, p < 0.001$ ; Block,  $F(5,110) = 4.55, p < 0.001$ ; Block x Lesion,  $F(5, 110) = 10.80, p < 0.001$ , figure 8.22).

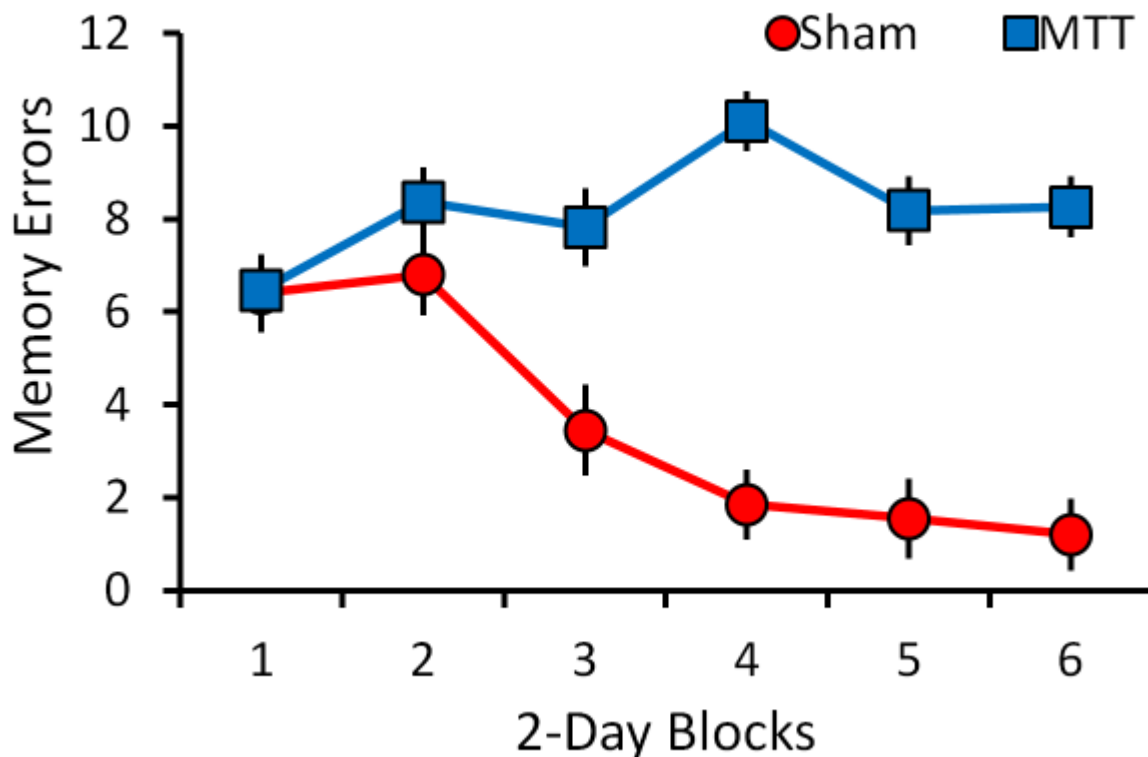
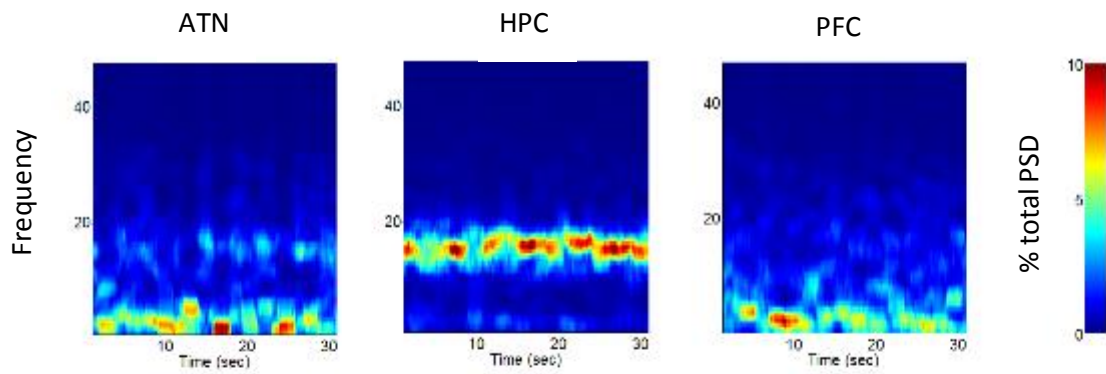


Figure 8.22: Mean  $\pm$  SEM spatial working memory errors on the standard radial arm maze task post-lesion surgery.

Sham

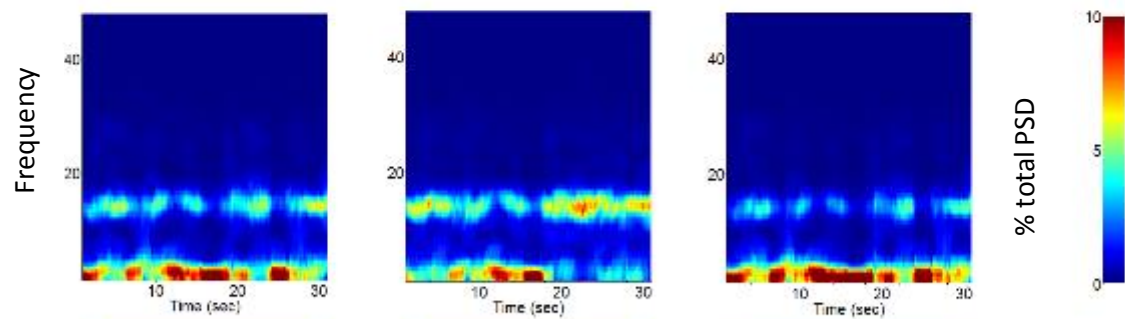
Baseline



Tail-pinch

MTT

Baseline



Tail-pinch

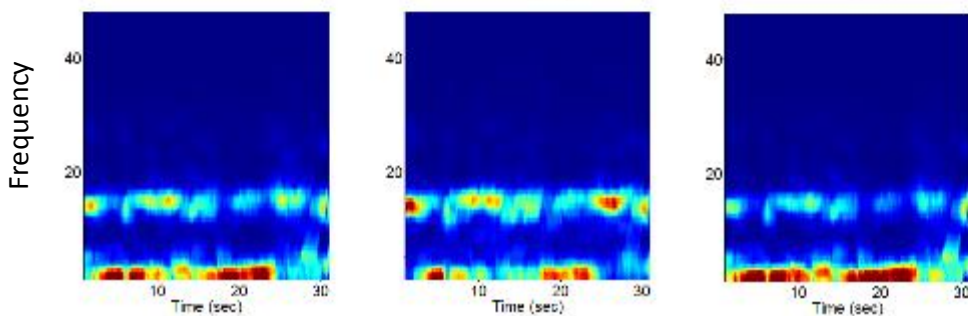


Figure 8.23: Spectrograms showing the relative power distribution (% of total power) in the ATN, HPC and PFC in a sham (top) and MTT rat (bottom) during baseline and tail pinch recordings under isoflurane anaesthesia. Warm colours indicate stronger power.

### 8.8.10. Baseline and tail pinch under isoflurane anaesthesia

#### 8.8.11. Power spectral density

A total of 60 seconds of electrophysiological signals per rat was analysed for each of the baseline and tail-pinch conditions. Baseline recordings made under isoflurane anaesthesia revealed a significant reduction in peak and total (Area) hippocampal power in the MTT group in the beta (Mann Whitney U (MWU): 27,  $Z = -2.22$ ,  $p = 0.024$  & MWU: 15,  $Z = -2.389$ ,  $p = 0.014$  and low gamma frequencies (MWU: 18,  $Z = -2.59$ ,  $p = 0.007$  & MWU: 21,  $Z = -2.38$ ,  $p = 0.014$ , figure 8.24c). The MTT group also had a reduction in high gamma peak power but not total (Area) in the hippocampus (MWU: 27,  $Z = -1.97$ ,  $p = 0.045$ ). No significant baseline differences were observed between the groups in the anterior thalamus or prefrontal cortex. The addition of a tail pinch, which is commonly used to drive theta rhythmicity in anaesthetised rats (Kocsis & Vertes, 2004) reduced both peak and total beta and low gamma power in the hippocampus of the sham group but had little effect in the MTT group (figure 8.24d). However, the MTT group still had a significant reduction in total beta power (MWU: 21,  $Z = -2.64$ ,  $p = 0.006$ ) and both peak and total low gamma power (MWU: 27,  $Z = -2.26$ ,  $p = 0.02$  & MWU: 24,  $Z = -2.45$ ,  $p = 0.01$ ) relative to shams. Like baseline recordings no differences were observed in the anterior thalamus or prefrontal cortex during tail-pinch.



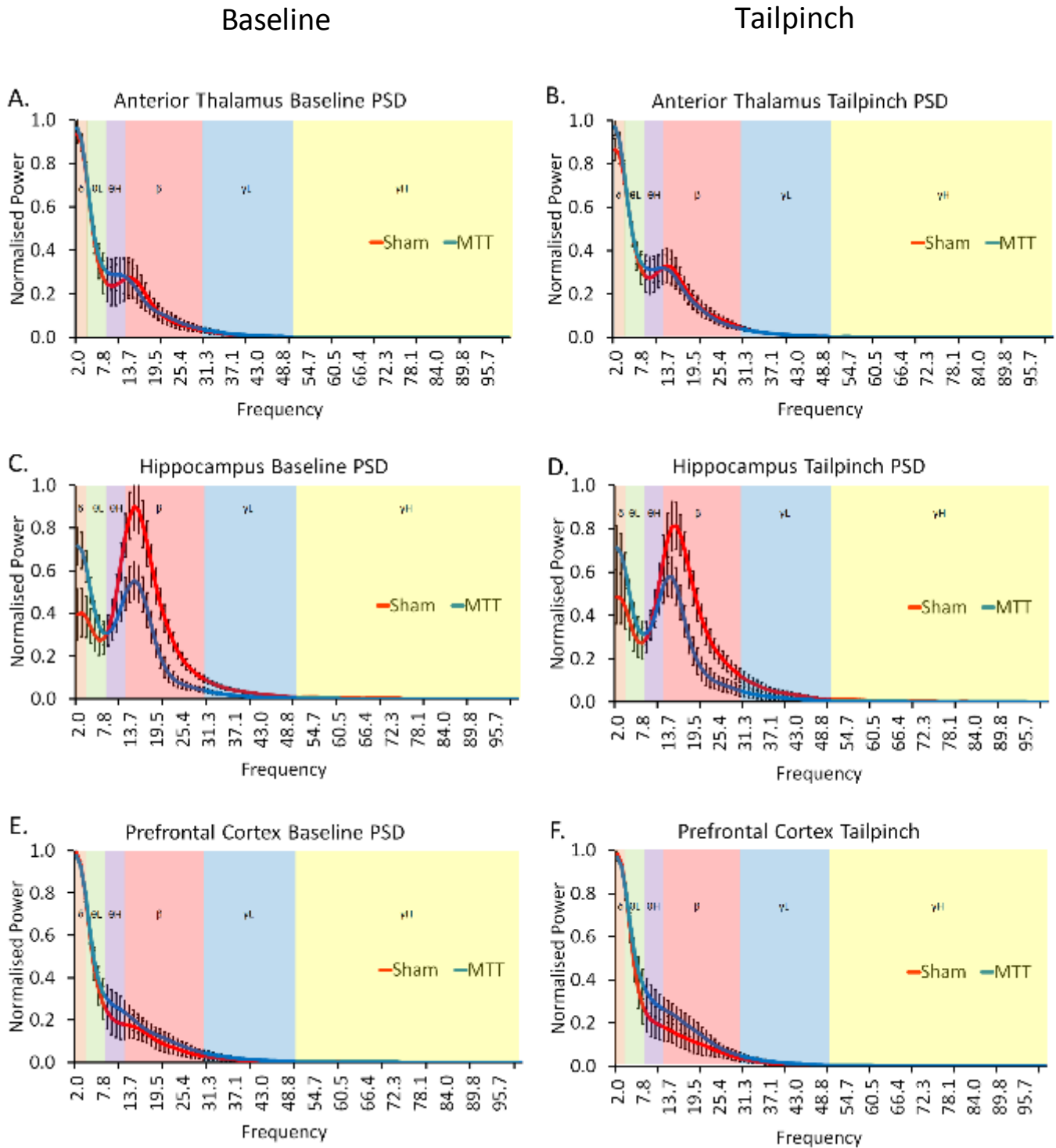


Figure 8.24: Mean  $\pm$  SEM power spectral densities from the ATN (A,B), hippocampus (C,D), and prefrontal cortex (E,F) during baseline (left panel) or tail pinch (right panel) under isoflurane anaesthesia.

### 8.3.12. Coherence

Differences in coherence between the HPC-ATN and between HPC-PFC, but not ATN-PFC were observed during baseline recording only (figure 8.25a,c). Rats with MTT lesions had less total HPC-ATN coherence in the beta band (MWU: 22,  $Z = -2.32$ ,  $p = 0.019$ ) and lower peak coherence in the low gamma band (MWU: 26,  $Z = -2.04$ ,  $p = 0.04$ ). Furthermore, the MTT lesion group had less total coherence between the HPC and PFC in the high theta band (MWU: 31,  $Z = -2.01$ ,  $p = 0.046$ ) and less peak and total coherence in the beta band (MWU: 31,  $Z = -2.01$ ,  $p = 0.046$ ; MWU: 30,  $Z = -2.07$ ,  $p = 0.04$ ). These differences were no longer apparent when a tail pinch was applied.

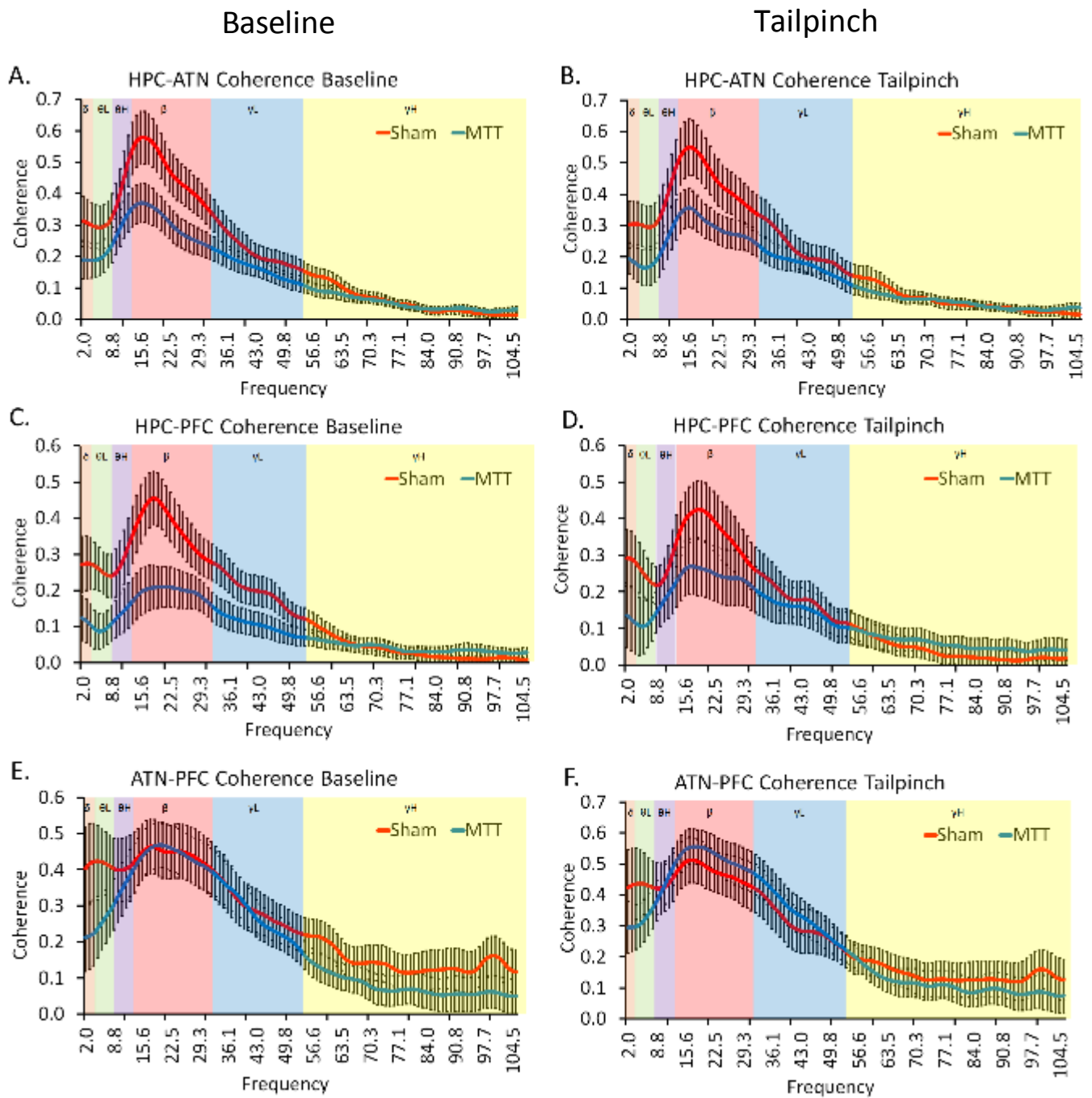


Figure 8.25: Mean  $\pm$  SEM coherence between the HPC-ATN (A,B), HPC-PFC(C,D) and ATN-PFC (E,F) during baseline (left panel) or tailpinch (right panel) under isoflurane anaesthesia.

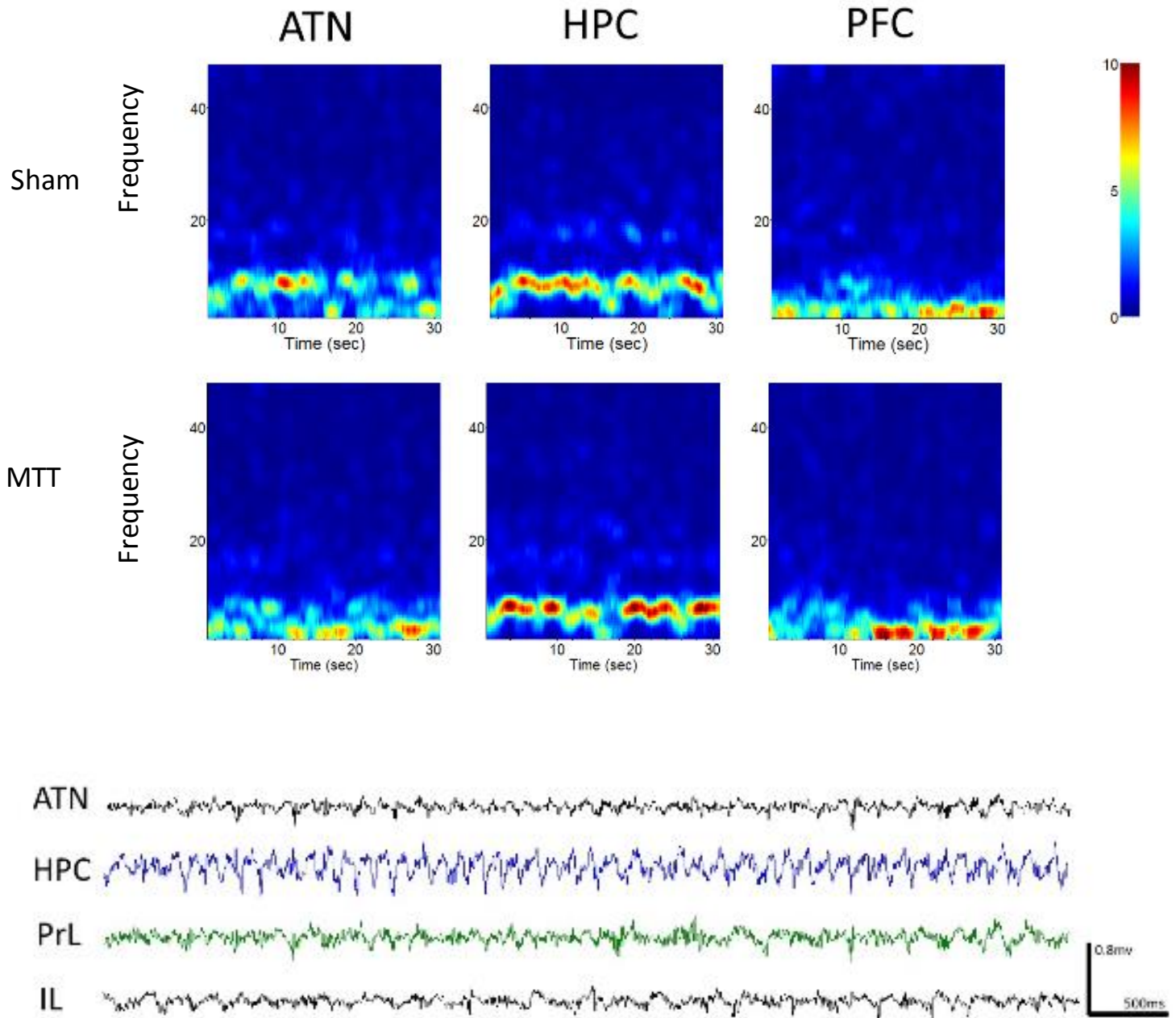


Figure 8.26: Spectrograms from the ATN, HPC and PFC of a Sham (top row) and MTT lesion rat (bottom row) during the random foraging task. An example of a raw LFP trace from the ATN, HPC prelimbic (PL; from one sham rat) and infralimbic (IL; from a different sham rat) during the random foraging task (below spectrograms).

### 8.3.13. Random Foraging in an openfield

Following the recovery period rhythmic oscillatory activity was examined in a random foraging task in a spatial environment (open field; see Methods) without any task specific demands. Data from the five 180 second daily sessions were pooled and analysed together for each rat. Example spectrograms and raw traces from a Sham rat and spectrograms from an MTT lesion rat are shown in figure 8.26. Behaviourally, no difference was found between MTT and sham rats in terms of velocity (sham,  $M = 7.45$  cm/s,  $SD = 1.18$ ; MTT,  $M = 6.95$  cm/s,  $SD = 1.96$ ,  $p = 0.48$ ) or total distance moved (sham,  $M = 2215.4$  cm/s,  $SD = 344.6$ ; MTT,  $M = 2070.5$ ,  $SD = 581.6$ ,  $p = 0.49$ ). This is important because rhythmic activity has been strongly associated with movement in rats (Young et al., 2009). No differences in power spectral densities were observed for the ATN, HPC or PFC across any frequency band or in terms of coherence between the HPC-ATN or HPC-PFC (figure 8.27). However, the MTT group had lower peak ATN-PFC coherence in the delta (MWU: 12,  $Z = -2.24$ ,  $p = 0.024$ ), theta (MWU: 14,  $Z = -2.04$ ,  $p = 0.042$ ) and low gamma bands (MWU: 11,  $Z = -2.34$ ,  $p = 0.018$ , figure 8.27f). The MTT group also had less total ATN-PFC coherence in the delta (MWU:14,  $Z = -2.04$ ,  $p = 0.039$ ), theta (MWU: 13,  $Z = -2.14$ ,  $p = 0.03$ ) and alpha bands (MWU: 10,  $Z = -2.44$ ,  $p = 0.012$ ).

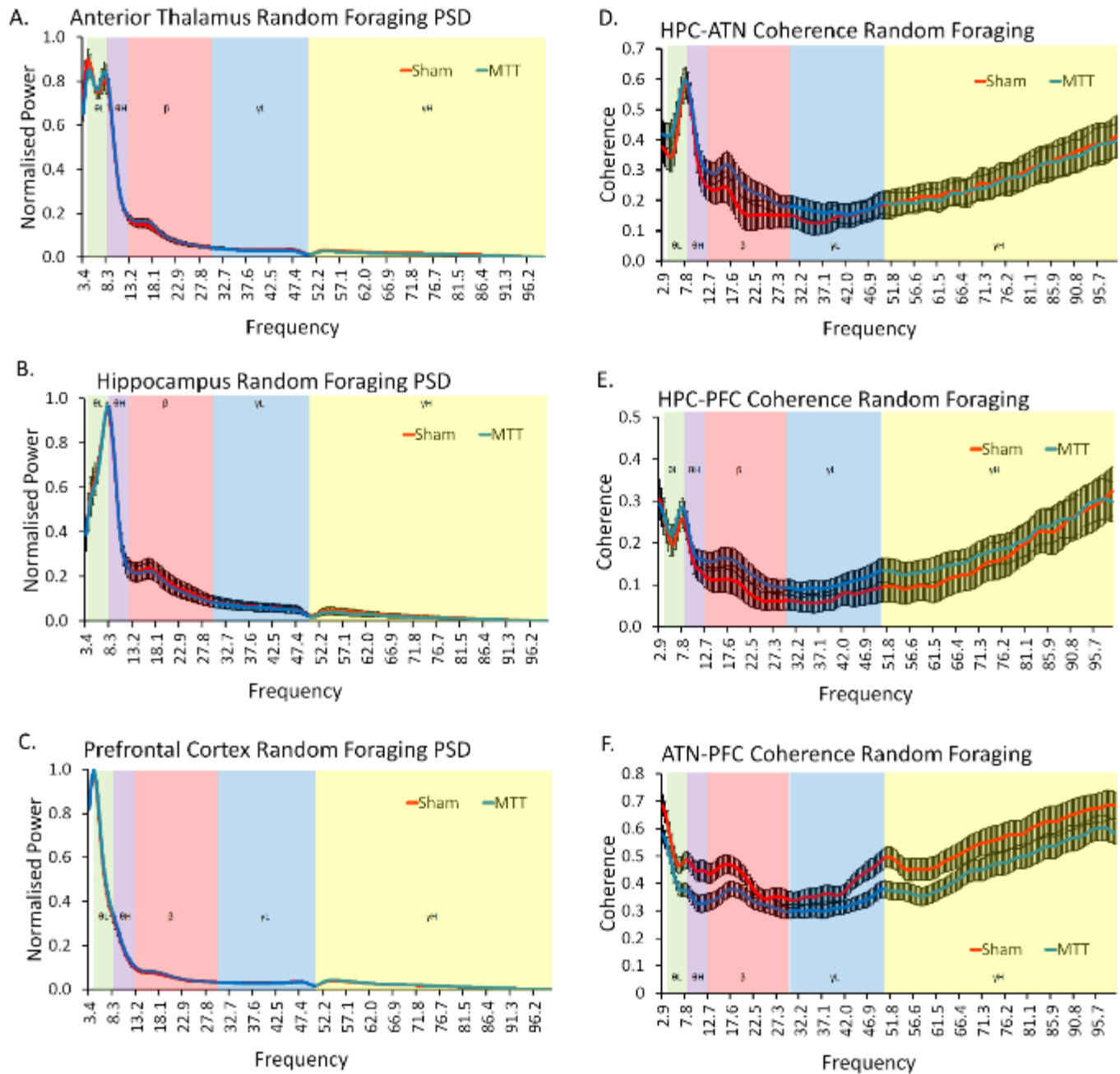


Figure 8.27: Mean  $\pm$  SEM power spectral densities (left panel) in the ATN (A), HPC (B) and prefrontal cortex (C) and coherence (right panel) between the HPC-ATN (D), HPC-PFC (E) during the random foraging task.

### 8.3.14. Spatial working memory in the RAM after electrode implantation

When rats were returned to the radial arm maze following electrode implantation the severe MTT lesion deficit was still evident (Lesion,  $F(1,22) = 55.31, p < 0.001$ ; figure 8.28). The Sham groups showed substantial reduction in spatial memory errors over time, but there was some evidence that the MTT group were also able to make mean improvements in errors over time (Block,  $F(5,110) = 7.23, p < 0.001$ ) but no (Block  $\times$  Lesion interaction,  $p > 0.1$ ).

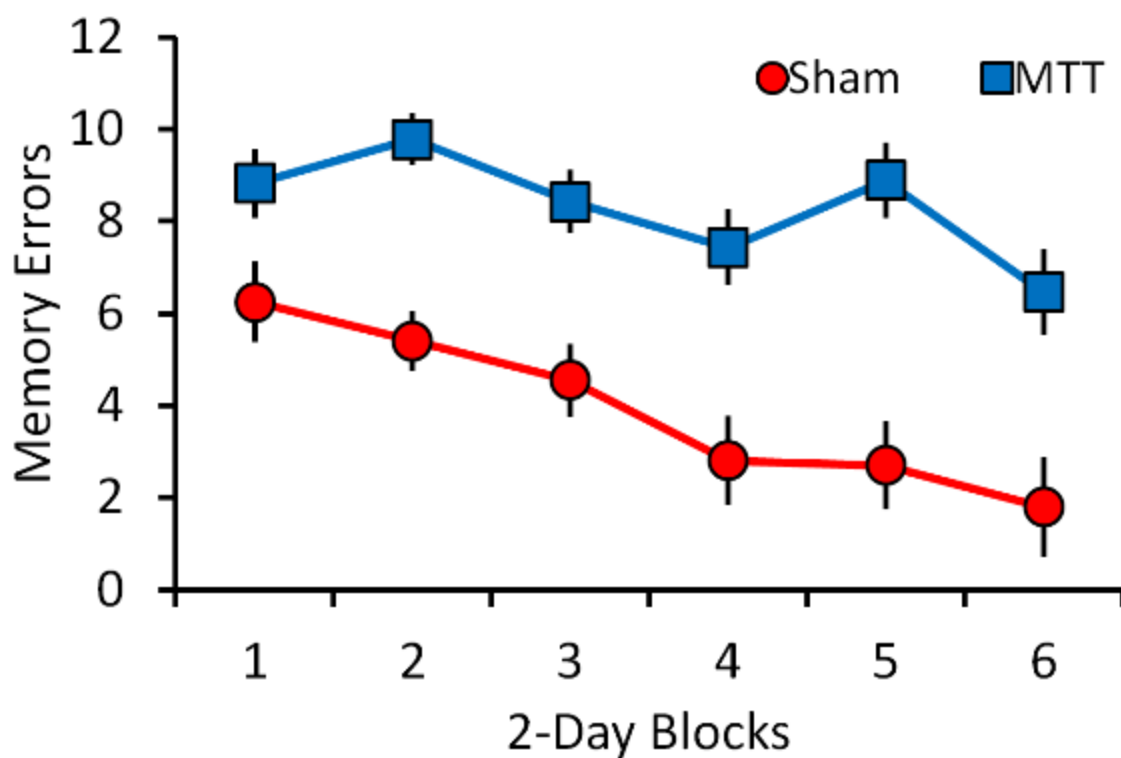


Figure 8.28: Mean  $\pm$  SEM Spatial working memory errors in the RAM across the 12 days of testing with recording

### 8.3.15. Electrophysiology during correct arm choices in the standard RAM

Correct arms were suited for the comparison between the MTT and Sham groups because the Sham group made relatively few errors. In this section recordings from 60 correct arm choice epochs per rat (60 sec) aggregated across the 12 days of testing were compared between the two groups. Rats with MTT lesions showed reduced peak and total high gamma power in the ATN during correct arm selection (MWU: 29,  $Z = -2.14$ ,  $p = 0.03$  & MWU: 30,  $Z = -2.07$ ,  $p = 0.041$ , figure 8.29a). Changes were also observed in the hippocampus where rats with MTT lesions had reduced high theta power (MWU: 31,  $Z = -2.01$ ,  $p = 0.045$ ) and reduced peak and total beta power (MWU: 24,  $Z = -2.45$ ,  $p = 0.012$  & MWU: 20,  $Z = -2.70$ ,  $p = 0.005$ , 8.29b). Coherence changes were only found between the ATN and PFC in the alpha band, where the MTT group had a substantial reduction in peak as well as total alpha coherence (MWU: 7,  $Z = -2.73$ ,  $p = 0.005$  & MWU: 9,  $Z = -2.53$ ,  $p = 0.008$ ).



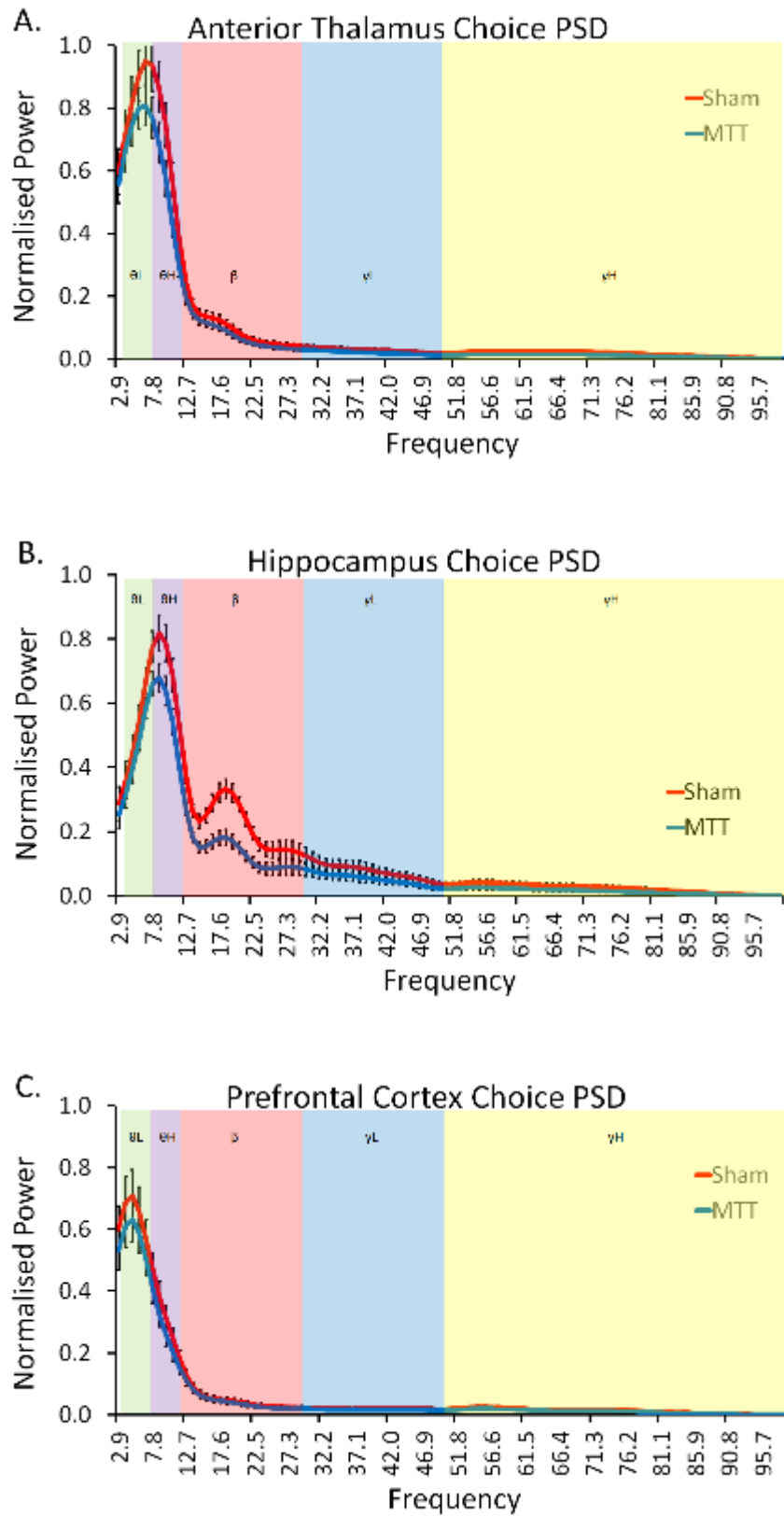


Figure 8.29: Mean  $\pm$  SEM power spectral density estimates in the ATN (A), HPC (B) and PFC (C) during correct arm selection.

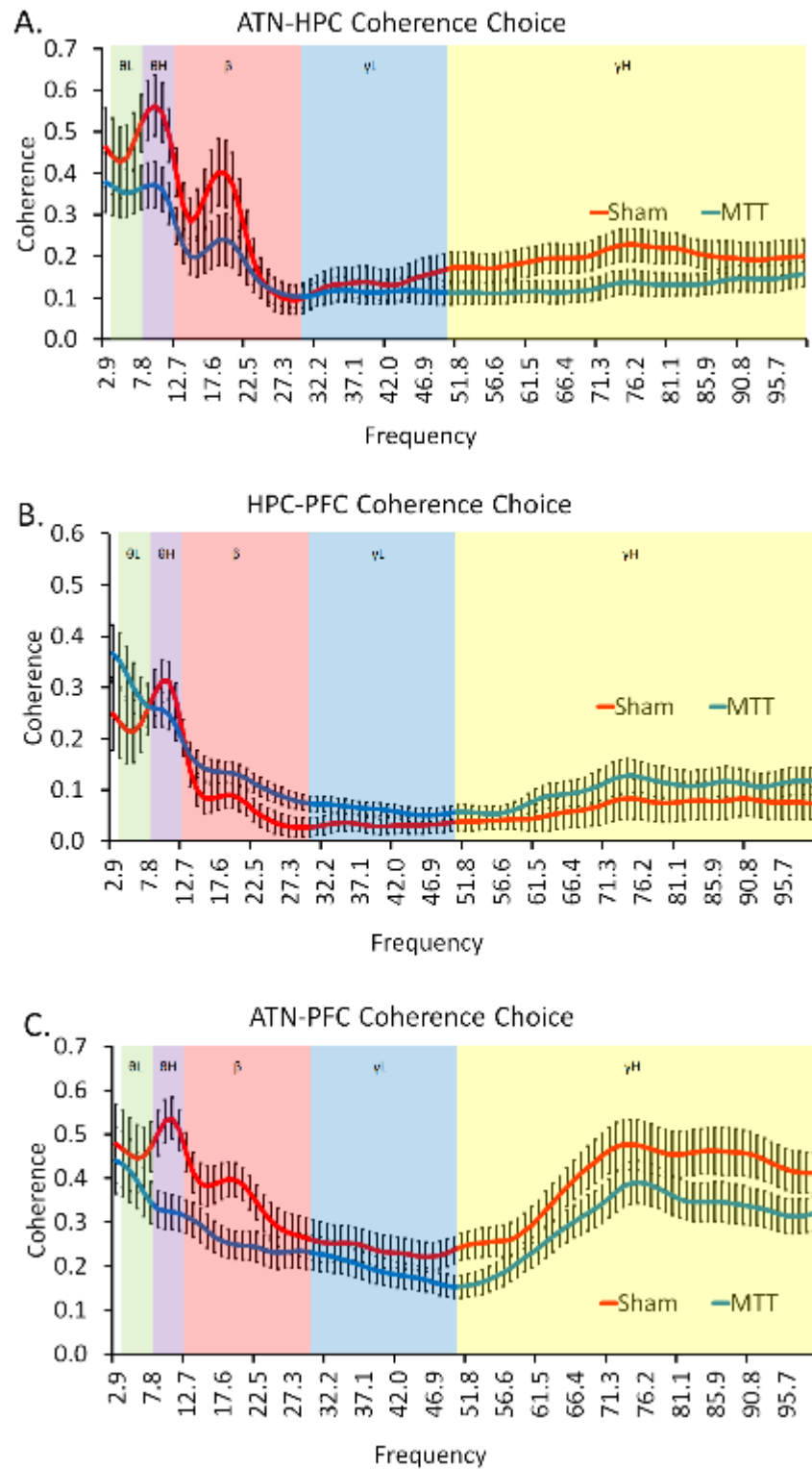


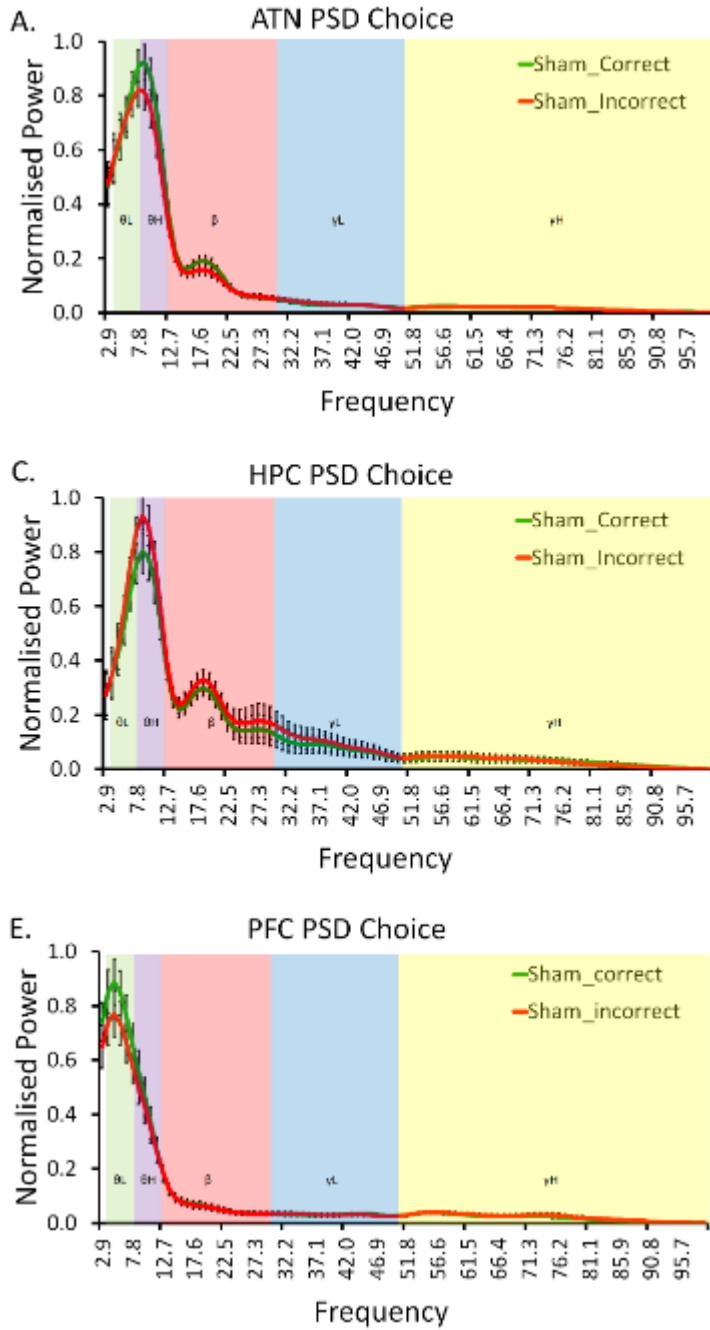
Figure 8.30: Mean  $\pm$  SEM coherence estimates between the HPC-ATN (A), HPC-PFC (B) and ATN-PFC (C) during correct arm selection.

### 8.3.16. Electrophysiology for correct vs incorrect arm choices in the standard RAM

Analysis of power and coherence between correct and incorrect arm choices revealed further differences between the sham and MTT groups. This was investigated using 21 (21 secs total) correct and incorrect trials per rat sampled across the 12 days of testing. This value was set by the low number of total spatial memory errors made by the sham group. The same patterns for power and coherence during correct choices within each group were present in this subset of the aforementioned data. Power differences between correct and incorrect trials in the sham group were restricted to the HPC and PFC (figure 8.31c,e), whereas the MTT group had power across all three structures with the strongest changes found in the ATN (figure 8.31b,d & f). The sham group showed decreased power for beta (Area:  $Z = -2.42, p = 0.012$ ) and low gamma (Peak & Area:  $Z = -2.66, p = 0.005$ ) in the hippocampus during correct arm choices. An additional increase was also observed in total high gamma power in the prefrontal cortex ( $Z = -2.29, p = 0.017$ ). By contrast, the MTT group showed decreased power in the alpha (Peak & Area:  $Z = -2.60, p = 0.007$  &  $Z = -2.79, p = 0.003$ ) and beta bands (Peak & AUC:  $Z = -2.73, p = 0.004$  &  $Z = -2.60, p = 0.007$ ) in the ATN during incorrect arm choices. The MTT lesion group also had greater peak low gamma power in the hippocampus ( $Z = -2.44, p = 0.013$ ) and increased high gamma peak and total power in the PFC (Peak & Area:  $Z = -2.16, p = 0.034$  &  $Z = -2.54, p = 0.009$ ) during incorrect arm choices.

More restricted changes were observed for coherence between correct and incorrect choices (figure 8.32). The sham group showed clear evidence of an increase in theta (Area:  $Z = -2.66, p = 0.004$ ) and alpha coherence (Peak & Area:  $Z = -2.66, p = 0.005$ ) between the hippocampus and PFC during correct vs incorrect trials (figure 8.32c). By contrast, The MTT group showed weaker coherence in the high gamma band (Peak & AUC:  $Z = -2.09, p = 0.038$ ) between the ATN and PFC for correct vs incorrect choices (figure 8.32f). stronger gamma coherence between the ATN and PFC was present for incorrect choices in the Sham group but did not reach significance ( $p = 0.1$ ).

## Sham



## MTT

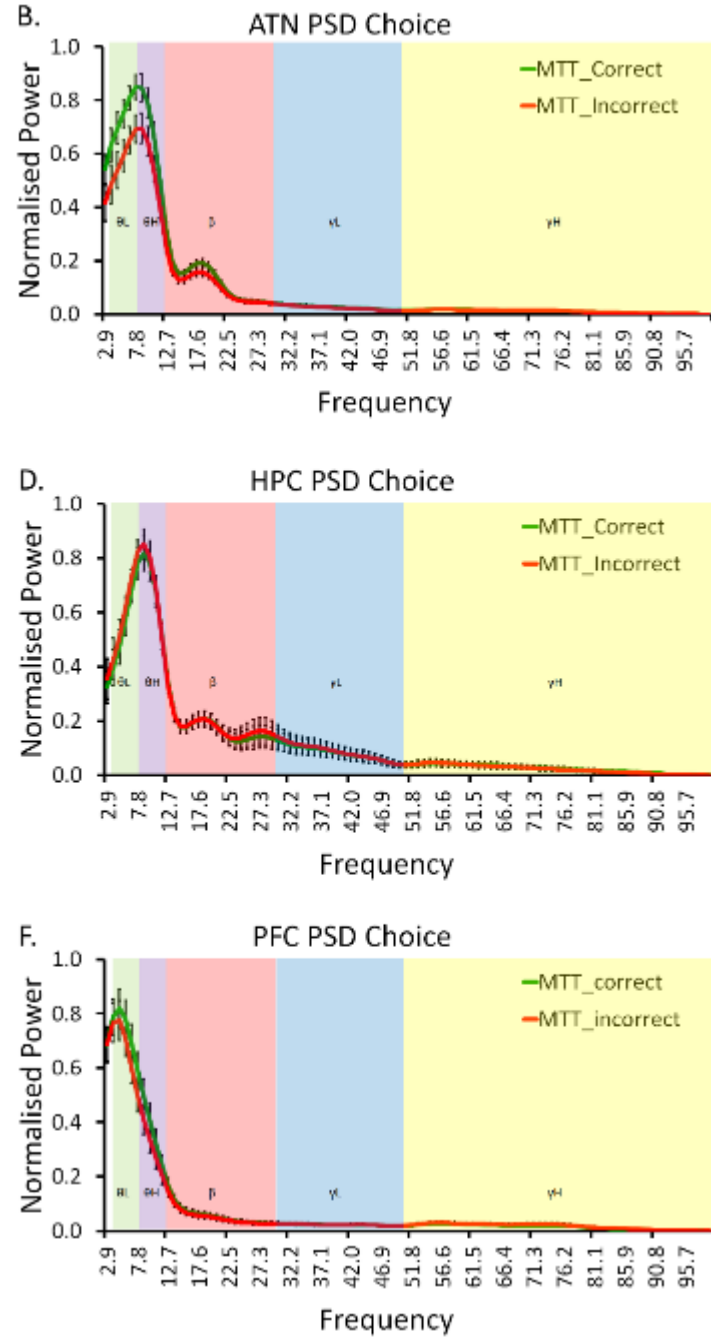


Figure 8.31: Mean  $\pm$  SEM estimated power spectral density in the ATN (A,B), HPC, (C,D) and PFC (E,F) for the sham (top panel) and MTT (bottom panel) groups across correct and incorrect choices in the standard radial arm maze task.

Sham

MTT

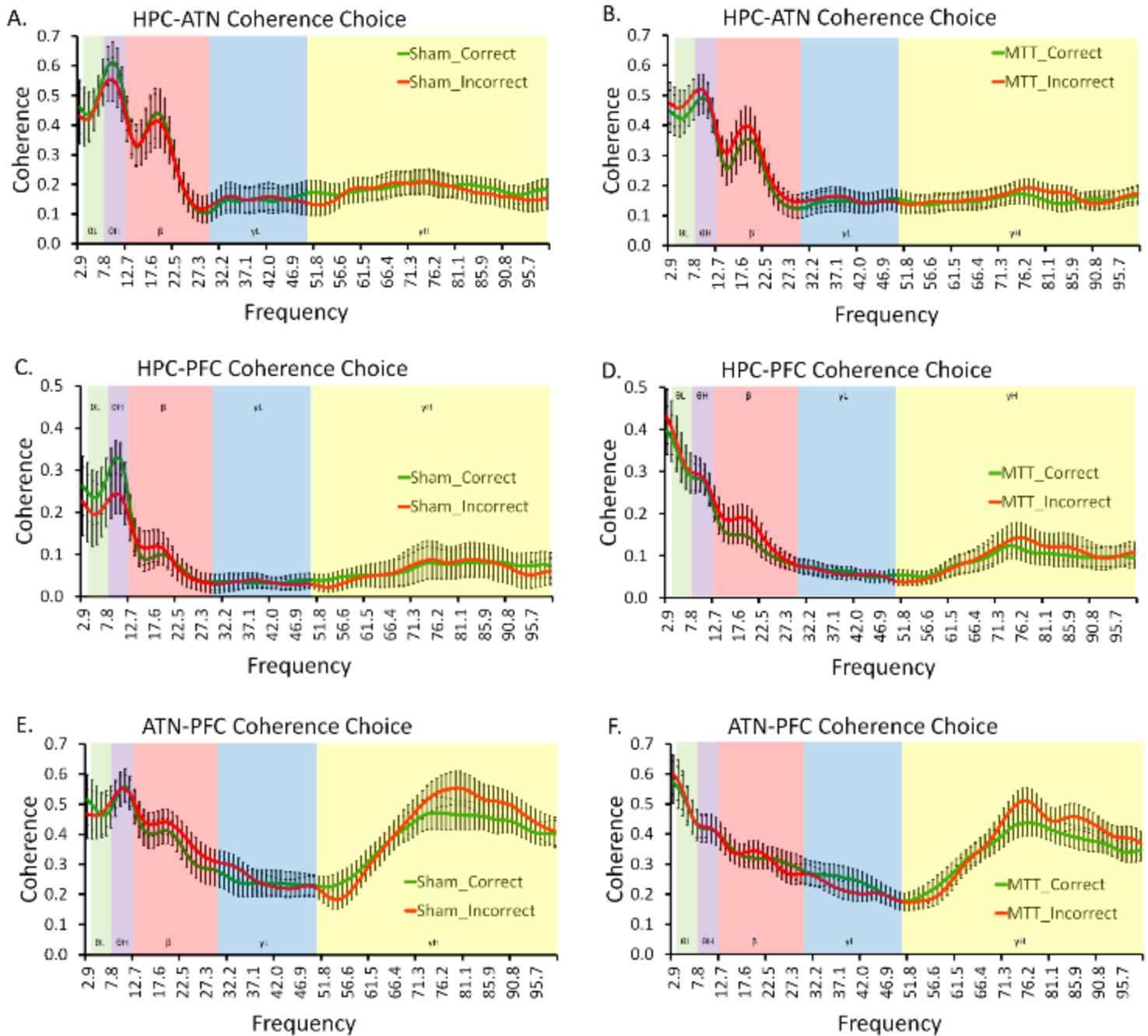


Figure 8.32: Mean  $\pm$  SEM estimated coherence between the HPC-ATN (A,B), HPC-PFC, (C,D) and PFC-ATN (E,F) for the sham (top panel) and MTT (bottom panel) groups across correct and incorrect choices in the standard radial arm maze task.

### 8.3.17. Mid-trial delay RAM task: Behaviour

To allow the comparison of relatively easy early arm selections made during early arm visits with the greater cognitive demands present in the later stages of the RAM task a five minute mid trial delay was added after the first four arm choices. Both groups were highly accurate during the first four arm choices and made minimal spatial memory errors (Sham mean = 0.01, SEM = 0.07; MTT mean = 0.25, SEM = 0.06). By contrast, the performance of both groups reduced substantially following the five minute mid trial delay (Trial Type,  $F(1, 22) = 120.07$ ,  $p < 0.001$ ; figure 8.33). Although this is not surprising as the pre-delay component ended when rats had made four arm choices, which restricted the number of errors they could make. Nonetheless, rats with MTT lesions made significantly more errors than shams after the delay (Trial Type x Lesion,  $F(1, 22) = 33.91$ ,  $p < 0.001$ ).

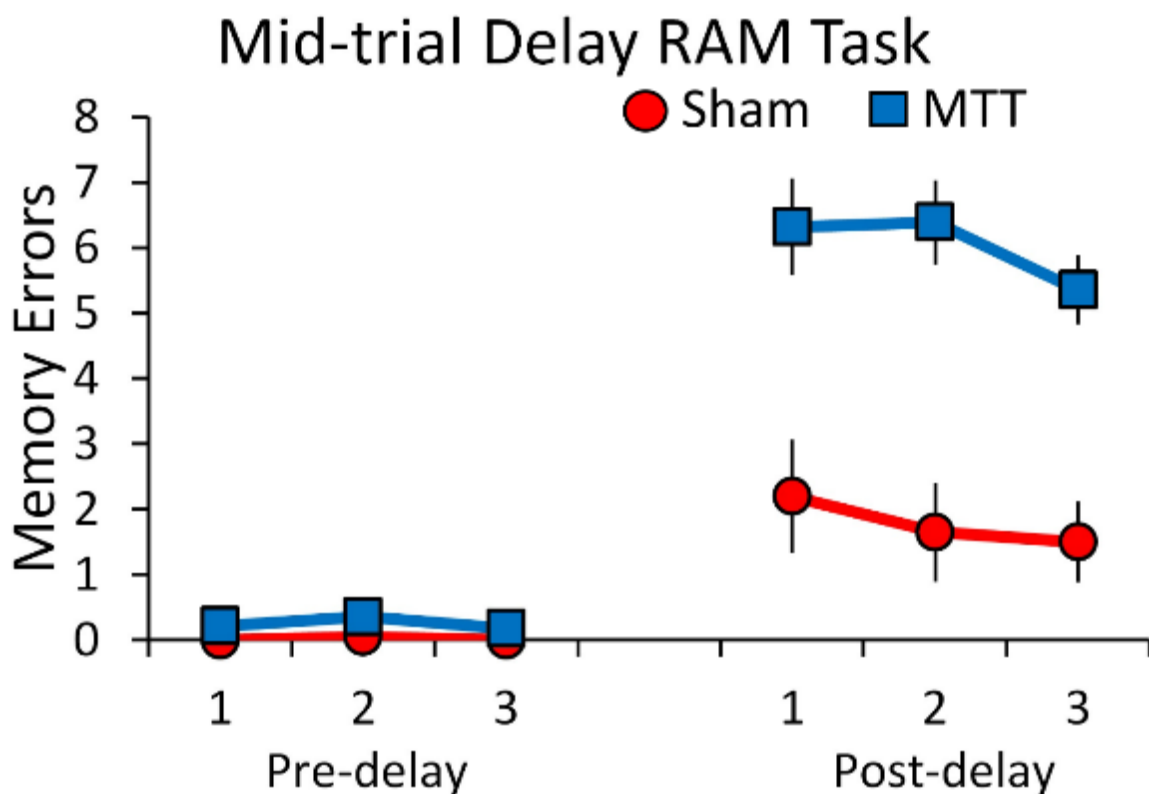


Figure 8.33: Mean  $\pm$  SEM working memory errors across the three 2 day blocks of testing in the mid trial delay ram task.

### 8.3.18. Correct arm choices in the mid trial delay RAM: Electrophysiology

To allow comparisons between pre and post delay performance 17 correct choice epochs per rat (17 seconds total) sampled across the six days of testing were aggregated and analysed for each of the pre and post delay blocks of choices. Due to time constraints only data from correct arm choices were analysed for this task. No differences in power between the MTT and sham group were found in the ATN, HPC and PFC either before or after the mid-trial delay (figure 8.34). However, the MTT group had reduced upper theta band coherence (8-12 Hz) across HPC-ATN, HPC-PFC and ATN-PFC both pre and post delay suggesting aberrant interactions were occurring both in the encoding and retrieval stages of the task. The strongest reductions in MTT coherence were observed in the upper theta band post delay between the HPC-ATN (Peak & Area MWU: 19,  $Z = -2.52$ ,  $p = 0.009$  & MWU: 14,  $Z = -3.08$ ,  $p = 0.001$ ), HPC-PFC (Peak & Area MWU: 20,  $Z = -2.70$ ,  $p = 0.006$  & MWU: 14,  $Z = -3.08$ ,  $p = 0.001$ ) and ATN-PFC (Peak & AUC MWU: 4,  $Z = -3.02$ ,  $p = 0.002$  & MWU: 4,  $Z = -3.02$ ,  $p = 0.001$ , figure 8.35b,d,f). Furthermore, reduced total coherence in the MTT group was observed in the high theta, beta and low gamma bands between the ATN-PFC (MWU: 14,  $Z = -2.04$ ,  $p = 0.042$ ; MWU: 13,  $Z = -2.14$ ,  $p = 0.03$ ; MWU: 13,  $Z = -2.14$ ,  $p = 0.03$ ) during the first four arm choices (figure 8.35e). Importantly, no differences in power or coherence were found between the MTT and sham group during the five minute delay when no task specific demands were present (figure 8.36).

## Pre-delay

## Post-delay

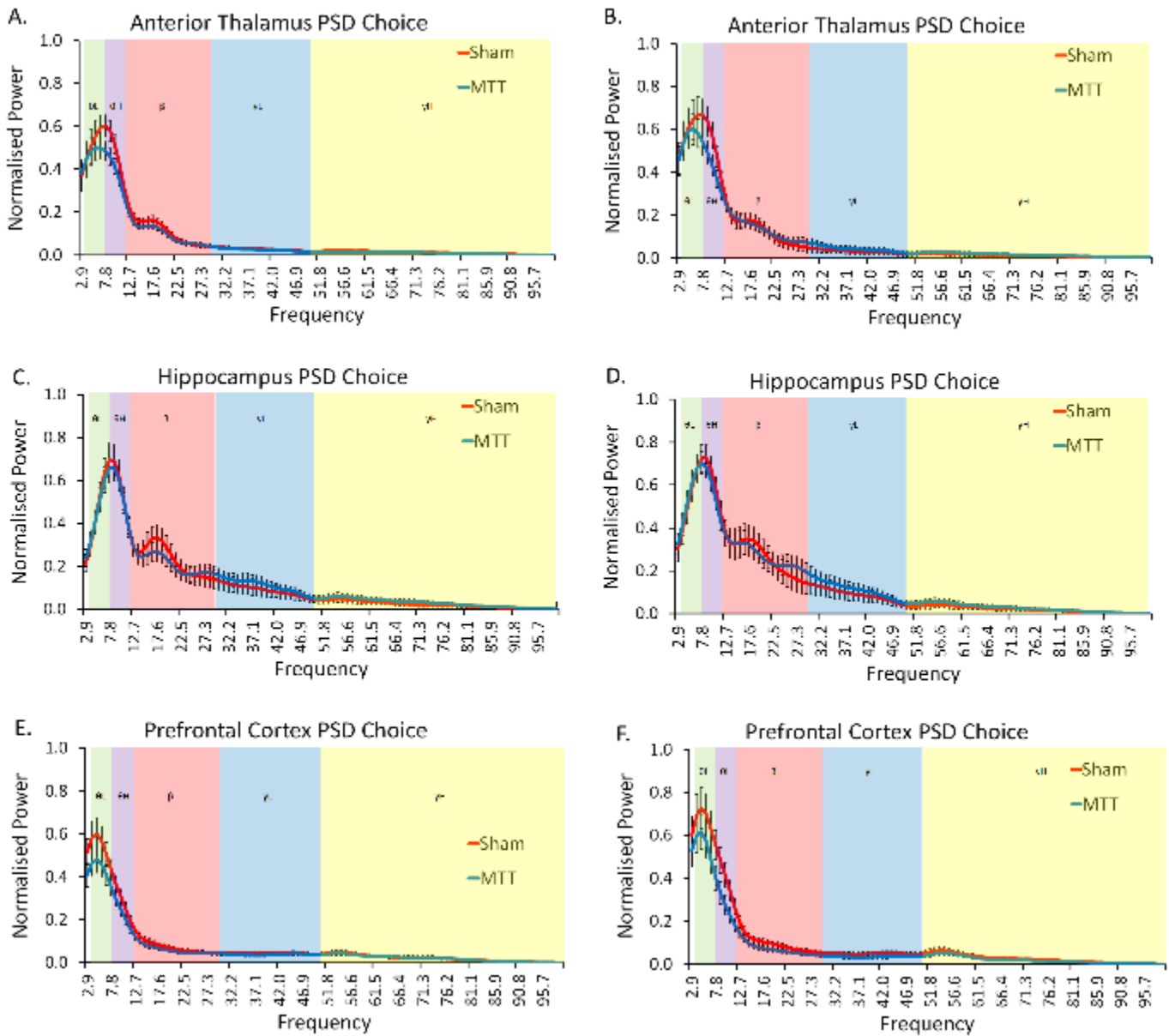


Figure 8.34: Mean  $\pm$  SEM estimated power spectral density in the ATN (A, B), HPC (C, D) and PFC (E, F), both prior to (left panel) and following (right panel) a five minute mid-trial delay.



## Pre-delay

## Post-delay

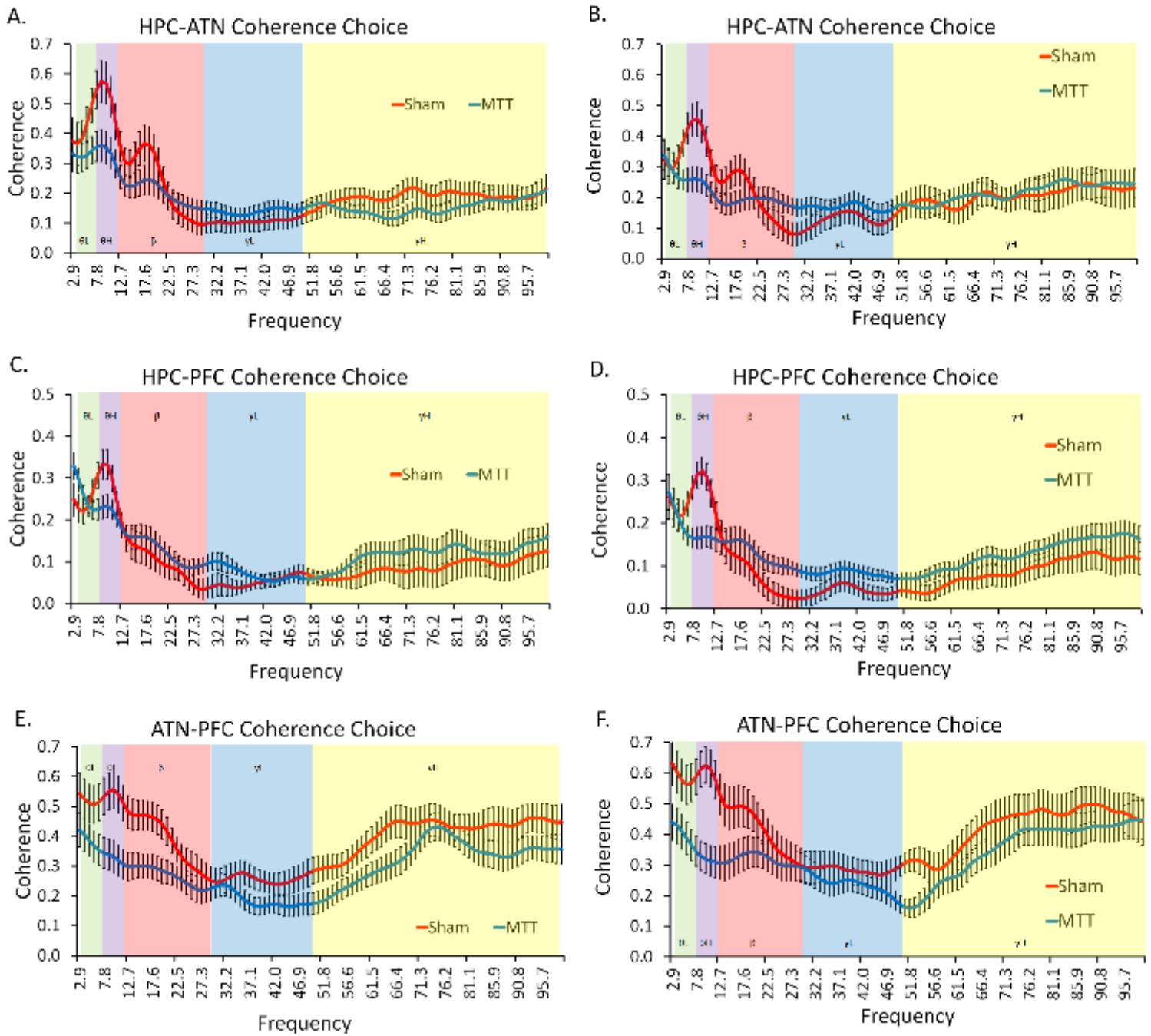


Figure 8.35: Mean  $\pm$  SEM estimated coherence between the HPC-ATN (A-B), HPC-PFC (C, D) and ATN-PFC (E,F), both prior to (left panel) and following (right panel) a five minute mid-trial delay.

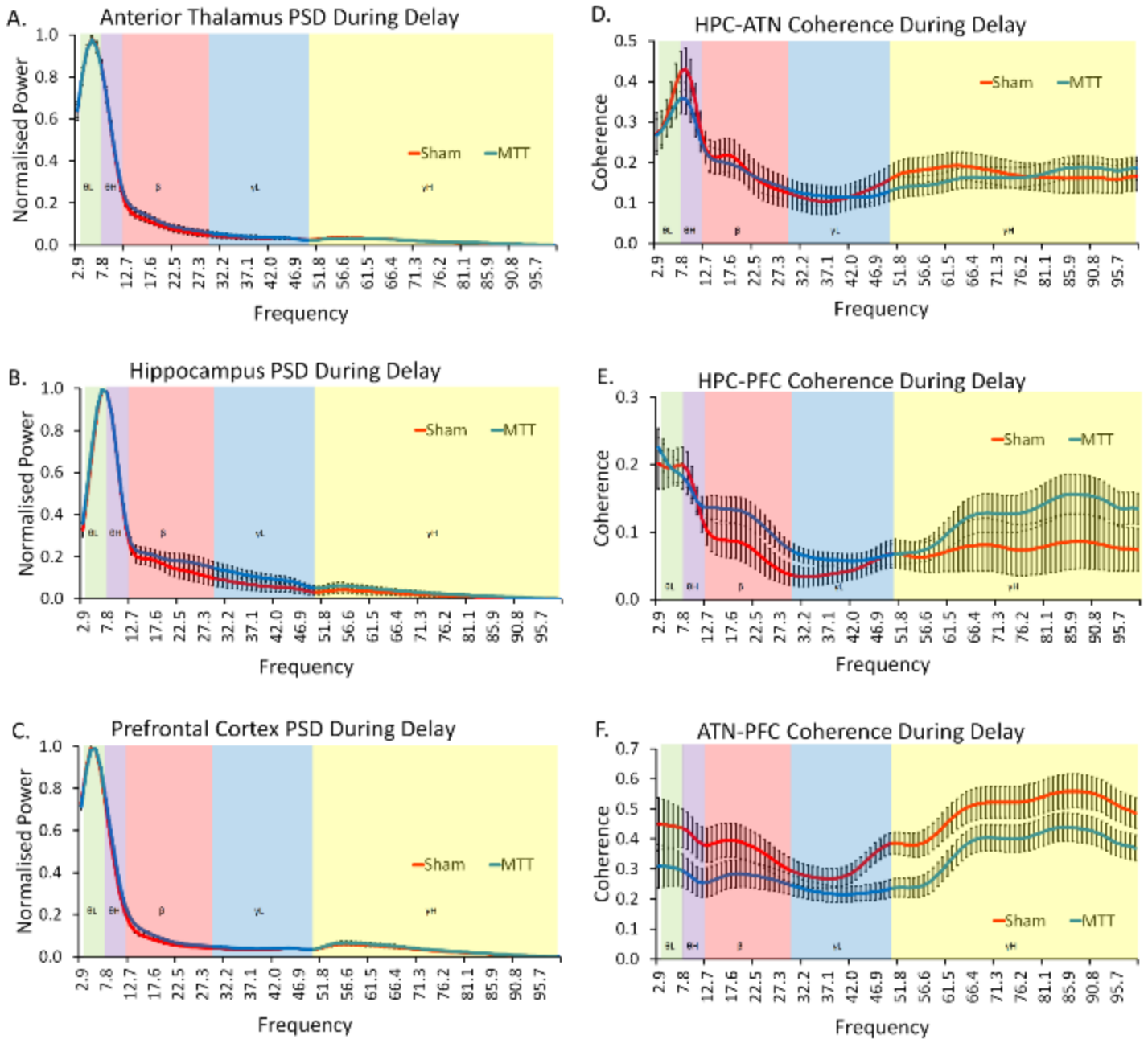


Figure 8.36: Mean  $\pm$ SEM power spectral density estimates (left panel) in the ATN (A), HPC (B) and the PFC (C) and coherence estimates (right panel) between the HPC-ATN (D), HPC-PFC (E) and ATN-PFC (F) during the five minute delay period.

### 8.3.19. Standard RAM with beam breaks: Behaviour

Following a break of 21 days, the rats were again run on the standard RAM task. This time beam breaks had been added down the arms of the RAM to increase accuracy of behaviour-electrophysiology associations. By this stage the sham group were showing consistently accurate levels of performance and minimal spatial memory errors even when they were first returned to the maze. Like before the MTT lesion group remained impaired relative to shams (Lesion,  $F(1,20) = 32.90$ ,  $p < 0.001$ ; figure 8.37) but now less than they had been during previous testing. Furthermore, the MTT group gradually improved across training blocks compared to the relatively stable performance in the sham group (Block,  $F(5,100) = 4.40$ ,  $p = 0.001$ ; Block  $\times$  Lesion,  $F(5,100) = 2.40$ ,  $p = 0.04$ ).

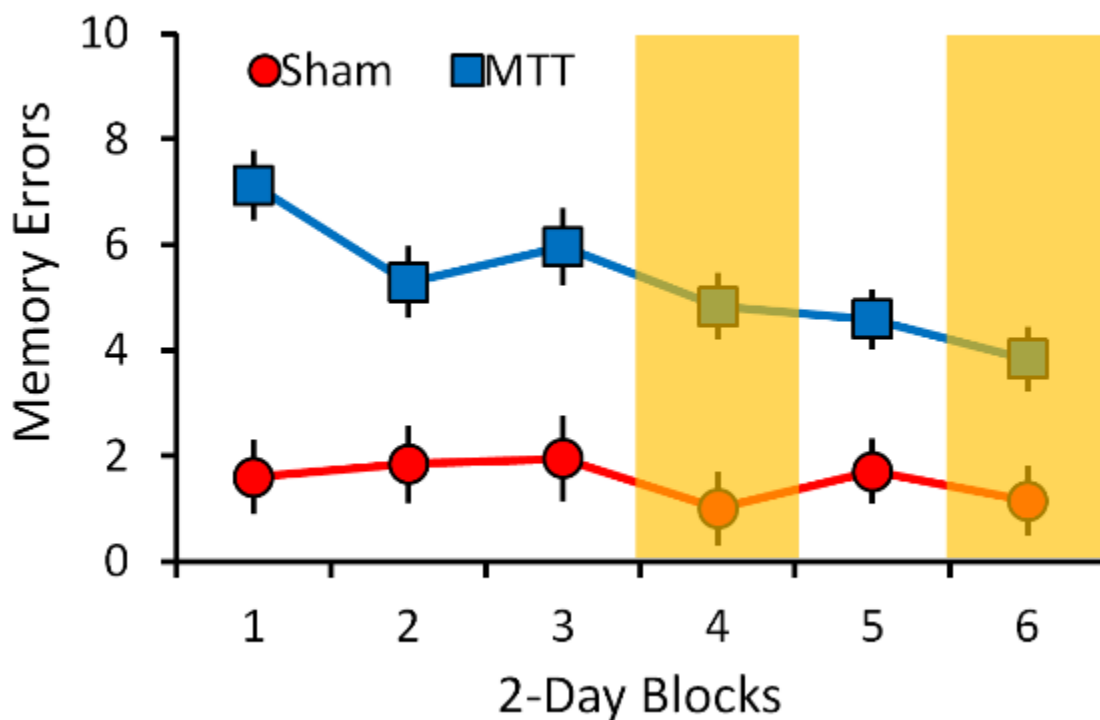


Figure 8.37: Mean  $\pm$  SEM spatial memory errors in the beam break RAM task across the 12 days of testing. Yellow blocks indicate the four days of neural recording.

### 8.3.20. Correct choices in the RAM (beam breaks): Electrophysiology

When the rats were returned to the standard working memory task in the radial arm maze electrophysiology differences between groups were more restricted than those found in the mid-trial delay task. Only four days of recording were made once the beam breaks had been added, so only 13 one second correct choice epochs from the first four and last four arm selections were analysed. Comparing between groups, rats with MTT lesions had increased peak power in the ATN in the low theta and high theta bands during the first four arm choices (figure 8.38a; MWU: 23,  $Z = -2.01$ ,  $p = 0.043$  & MWU: 18,  $Z = 1.8$ ,  $p = 0.013$ ) along with increased total power in the high theta band (MWU: 21,  $Z = -2.16$ ,  $p = 0.027$ ). Furthermore, the MTT group had an increase in total theta power in the hippocampus in both the first and last four arm choices (MWU: 17,  $Z = -2.62$ ,  $p = 0.008$  & MWU: 10,  $Z = -3.12$ ,  $p = 0.001$ , figure 8.38c, d). Reduced coherence was observed in the MTT group but only between the ATN-PFC (figure 8.39e, f). In the MTT group reduced coherence between the ATN-PFC were found for peak and total beta coherence (MWU: 6,  $Z = -2.32$ ,  $p = 0.021$  & MWU: 8,  $Z = -2.06$ ,  $p = 0.043$ ) and total low gamma coherence (MWU: 8,  $Z = -2.06$ ,  $p = 0.043$ ) in the first four arm choices, and reductions in peak and total high theta coherence in the last four arm choices (MWU: 6,  $Z = -2.32$ ,  $p = 0.021$ ; MWU: 7,  $Z = -2.19$ ,  $p = 0.03$ ).

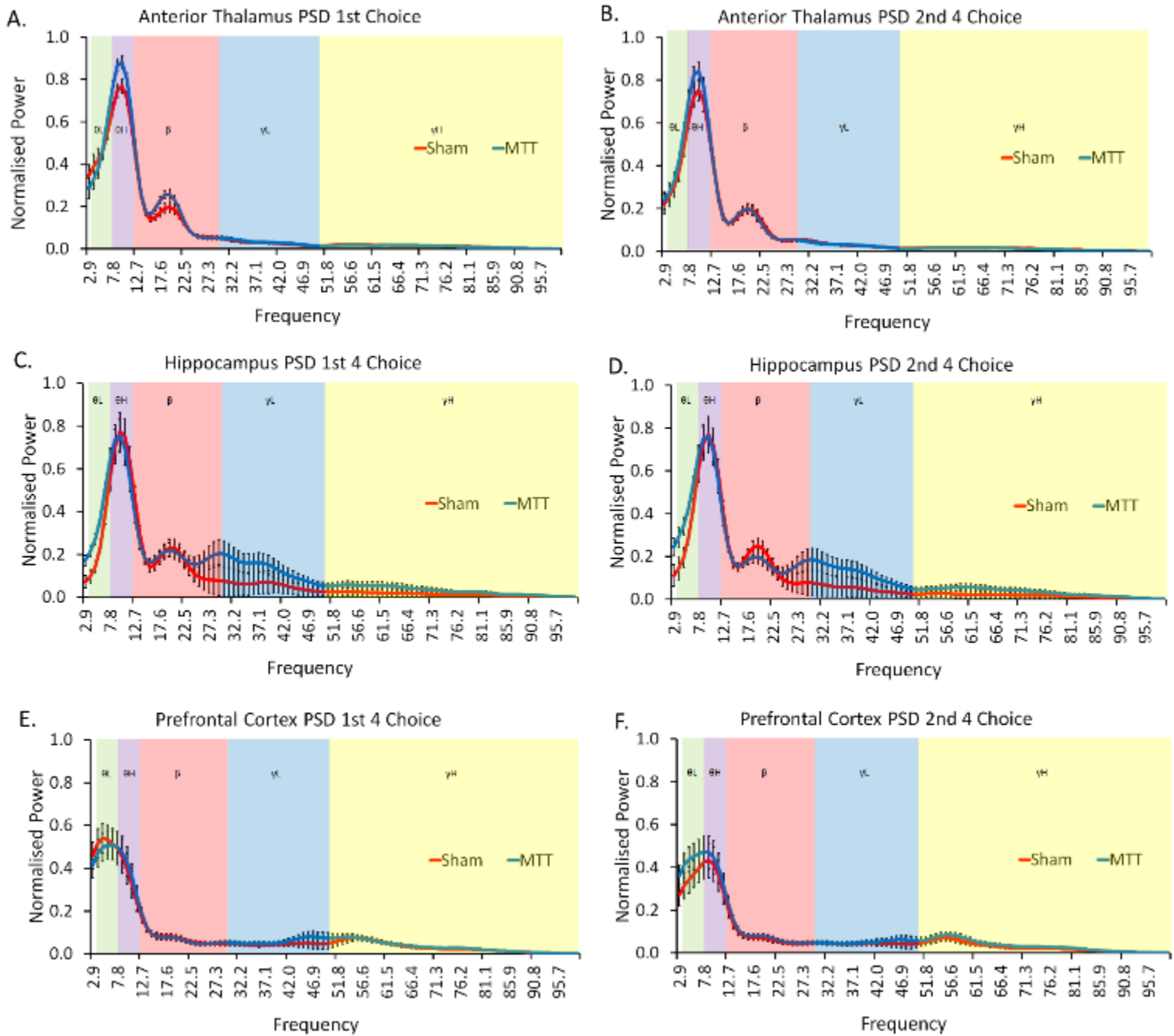


Figure 8.38: Mean  $\pm$  SEM power spectrum density estimates in the ATN (A,B), HPC (C, D) and PFC (E,F) during the first four (left panel) and last four (right panel) correct arm choices.

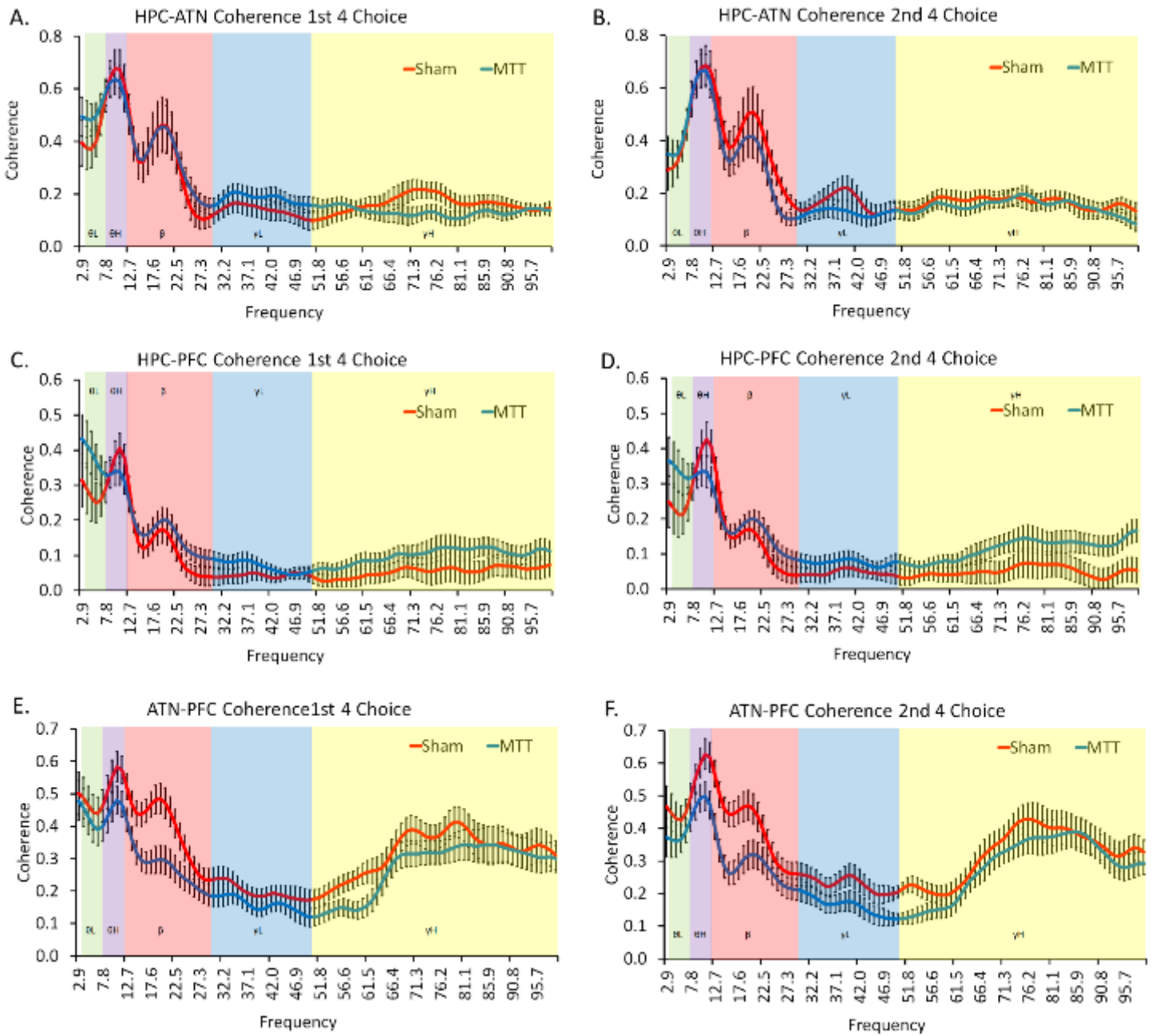


Figure 8.39: Mean  $\pm$  SEM coherence estimates between the HPC-ATN (A,B), HPC-PFC (C, D) and ATN-PFC (E,F) during the first four (left panel) and last four (right panel) correct arm choices.

### 8.3.21. Incorrect choices in the standard RAM (beam breaks) - electrophysiology

Only eight of the eleven remaining MTT rats made sufficient errors to compare correct and incorrect trials on this task (13+); none of the sham rats made sufficient incorrect arm choices. That is, 13 one second epochs from incorrect arm choices were analysed for the last four arms in the MTT group only. Rats with MTT lesions showed an increased total and peak alpha power in the ATN during an correct arm selection ( $Z = -2.38, p = 0.016$  &  $Z = -2.38, p = 0.014$ ; figure 3.40a) along with decrease in total power in hippocampal beta ( $Z = -2.52, p = 0.008$ , 3.40b figure) and prefrontal high gamma power ( $Z = -2.10, p = 0.037$ , figure 3.40c). Changes in coherence were only found between the HPC-PFC (figure 3.40e). Higher peak beta coherence ( $Z = -2.52, p = 0.008$ ) and a decrease in total high gamma coherence ( $Z = -2.52, p = 0.008$ ) were associated with correct choices.



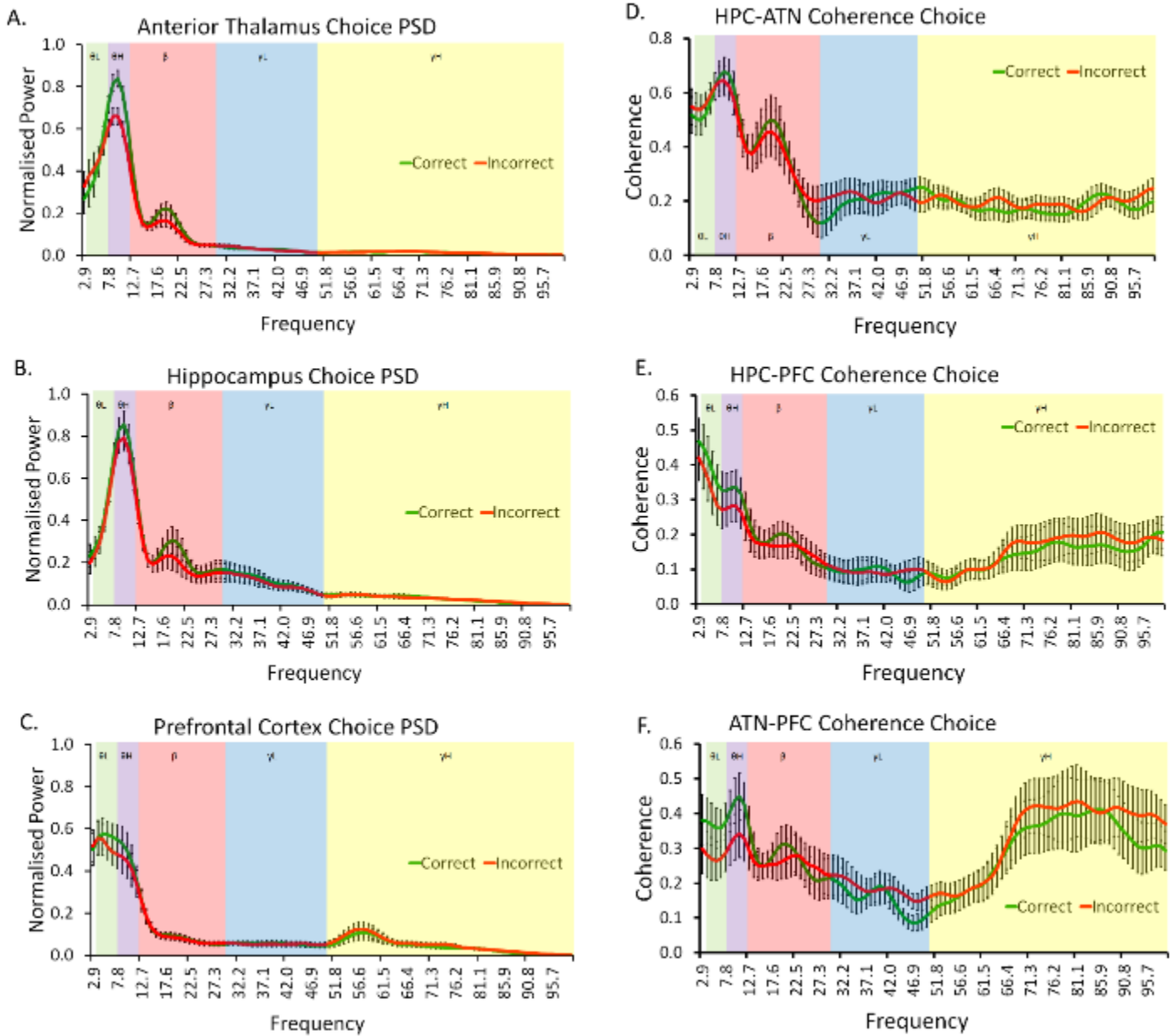


Figure 8.40. Mean  $\pm$  SEM power spectral density estimates (left panel) in the ATN (A) HPC (B) and PFC (C) and coherence (right panel) between the HPC-ATN (A) HPC-PFC (B) and ATN-PFC (C) for correct and incorrect choices in the standard RAM task with beam breaks.



### 8.3.23. Theta-gamma cross-frequency coupling

As discussed in chapter 5 growing evidence has suggested an important role for theta-gamma interactions in mnemonic processes. Therefore, theta-gamma cross-frequency-coupling was examined in the hippocampus during the first four correct arm choices in the beambreak RAM task. The degree to which low and high theta phase was related to low and high gamma amplitude was determined using the modulation index (MI; from Tort et al., 2010). No differences between the sham and MTT groups were found for low theta-low gamma, low theta-high gamma or high theta-low gamma phase amplitude interactions in the hippocampus (figure 8.41). However, the sham showed significantly greater modulation of high-theta phase high gamma amplitude interactions (MWU: 20,  $Z = -2.241$ ,  $p = 0.025$ ; figures 8.41 & 8.42).

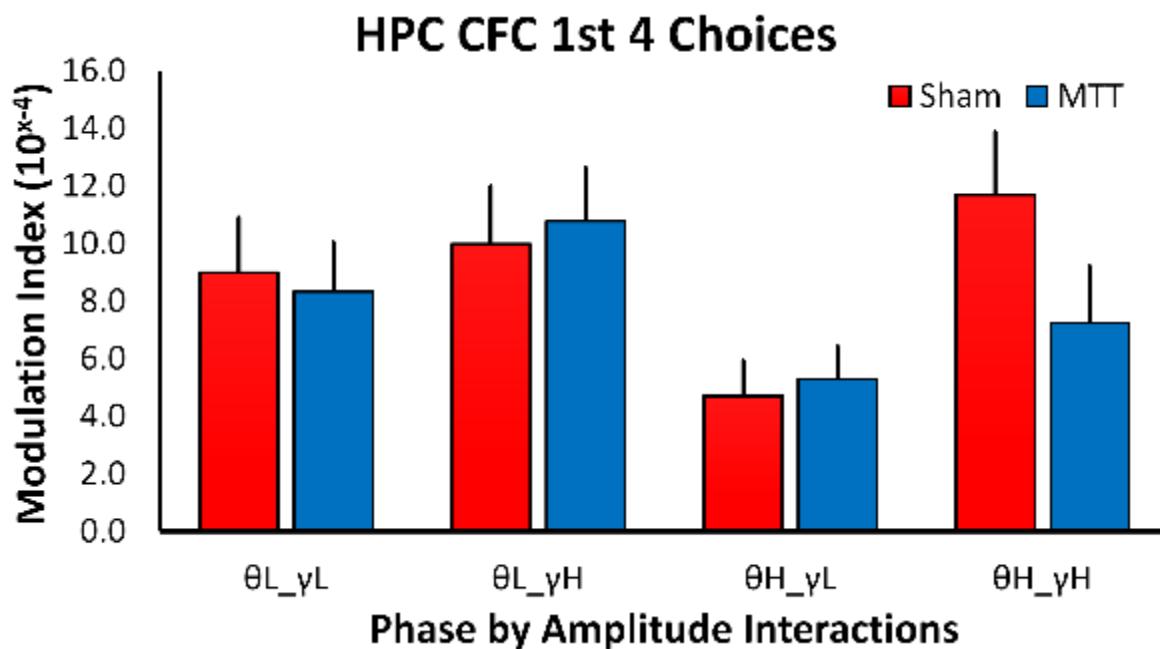


Figure 8.41: Mean + SEM modulation index between the phase of low and high theta and the amplitude of low and high gamma.  $\theta L_{\gamma L}$  = low theta phase (4-7.9 Hz) vs low gamma amplitude (30-48 Hz);  $\theta L_{\gamma H}$  = low theta phase (4-7.9 Hz) vs high gamma amplitude (52-100 Hz);  $\theta H_{\gamma L}$  = high theta phase (8-12 Hz) vs low gamma amplitude;  $\theta H_{\gamma H}$  = high theta phase vs high gamma amplitude.

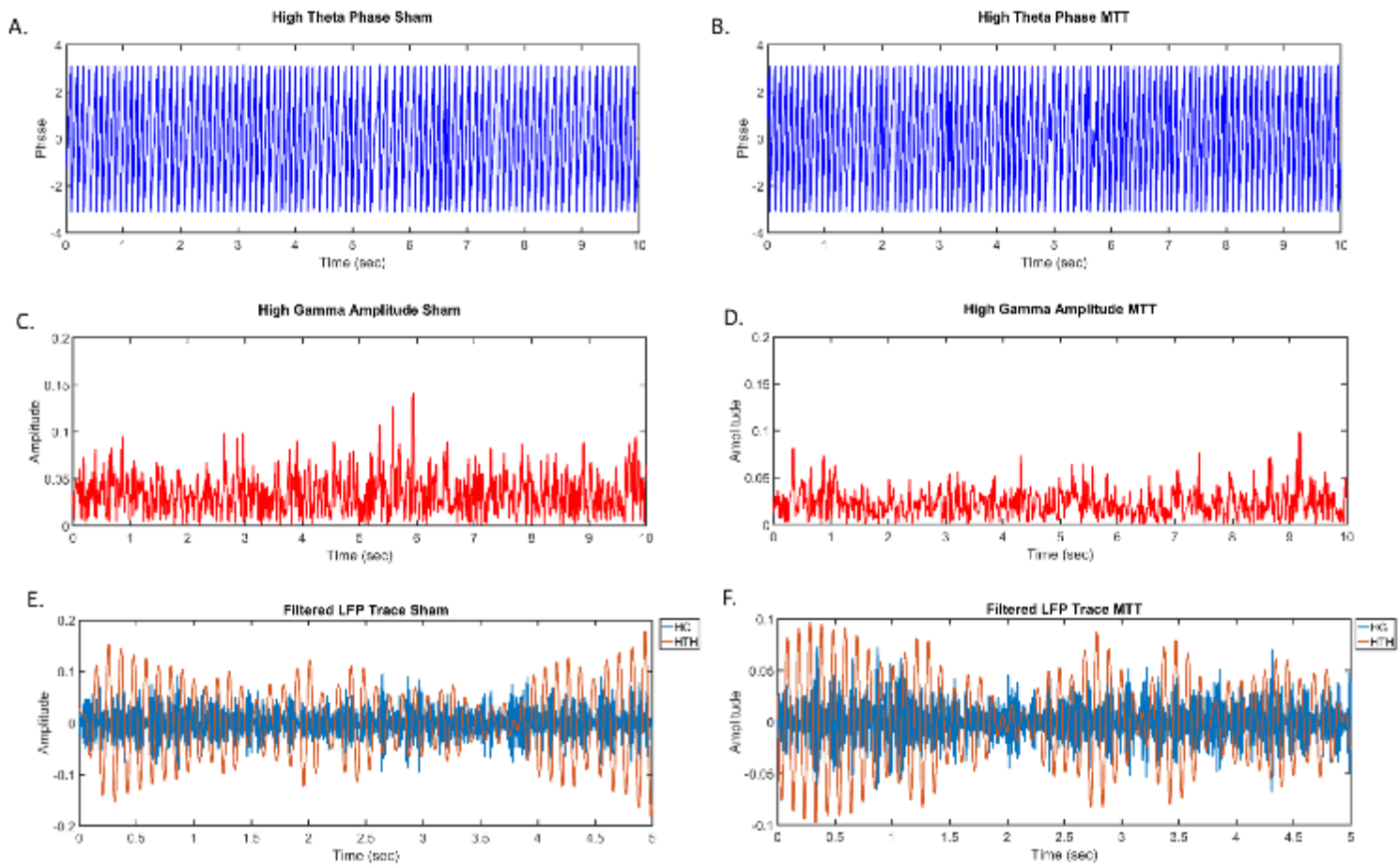


Figure 8.42: Examples of high theta phase from a sham (A) and MTT (B) rat and high gamma amplitude from the same sham (C) and MTT rat (D). The bottom two panels show filtered LFP traces of high gamma (blue) superimposed on high theta oscillations (orange) in the sham (E) and MTT rat (F).

### 8.3.23. TBS of the ATN in a holding cage

A secondary aim of this experiment was to examine the efficacy of theta burst stimulation (TBS) of the ATN to enhance memory function. The initial phase of this was to examine the effect of TBS on power and coherence within and between the ATN, HPC and PFC in a standard holding cage. The five seconds preceding (Pre TBS) and subsequent to (Post TBS) one second trains of bilateral theta burst stimulation to the ATN were aggregated and analysed for each rat (25 seconds for each epoch). TBS produced an increased peak in low theta power in the prefrontal cortex of the sham group ( $Z = -2.20$ ,  $p = 0.33$ , figure 8.43c) and a close to significant increase in peak high theta power in the MTT group ( $Z = -1.96$ ,  $p = 0.055$ , figure 8.43d). In terms of HPC-PFC coherence TBS decreased total delta coherence ( $Z = -2.10$ ,  $p = 0.039$ ) and increased total low theta coherence ( $Z = -2.10$ ,  $p = 0.039$ ) in the sham group (figure 8.43e), but reduced peak high theta coherence in the MTT group ( $Z = -2.31$ ,  $p = 0.021$ , figure 8.43f).

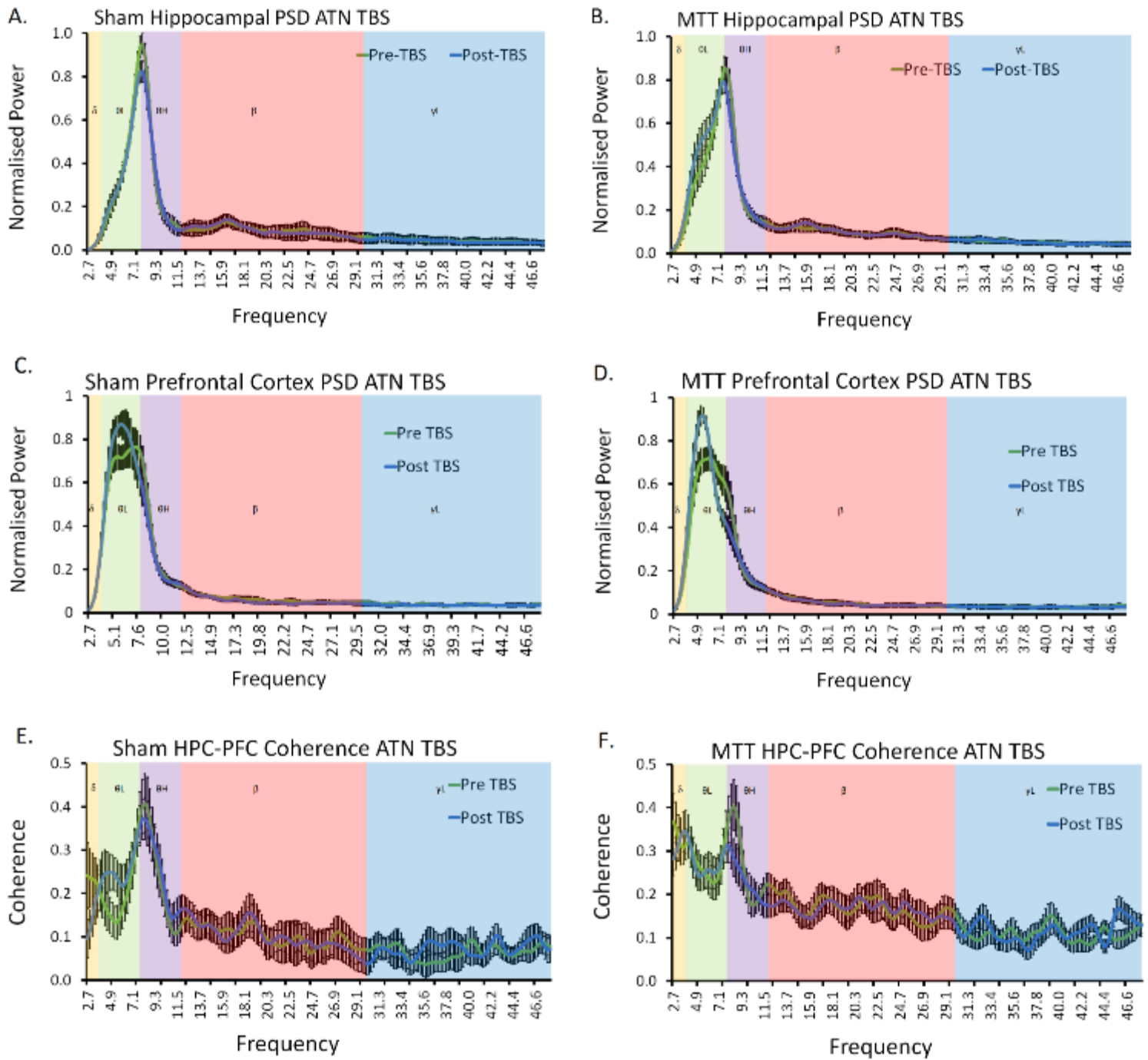


Figure 8.43: Mean  $\pm$  SEM power spectrum density estimates in the HPC (A,B), and PFC, (C,D) and HPC-PFC coherence (E,F) for the sham (left panel) and MTT (right panel) groups for the five seconds preceding (Pre TBS) and five seconds subsequent to (Post TBS) TBS stimulation.

### 8.3.24. ATN TBS stimulation during RAM

TBS was then applied during the first four arm choices in the standard RAM task to determine its effects on spatial memory across five days of testing. Rats were limited to a maximum of 10 arm entries for this task, which reduced the number of possible errors. In line with previous testing the MTT group made significantly more errors than sham rats (Lesion,  $F(1,19) = 32.52$ ,  $p < 0.001$ ; figure 8.44). However, neither group's performance was significantly affected by TBS of the ATN (Trial Type and Trial Type x Lesion  $p > 0.3$ )

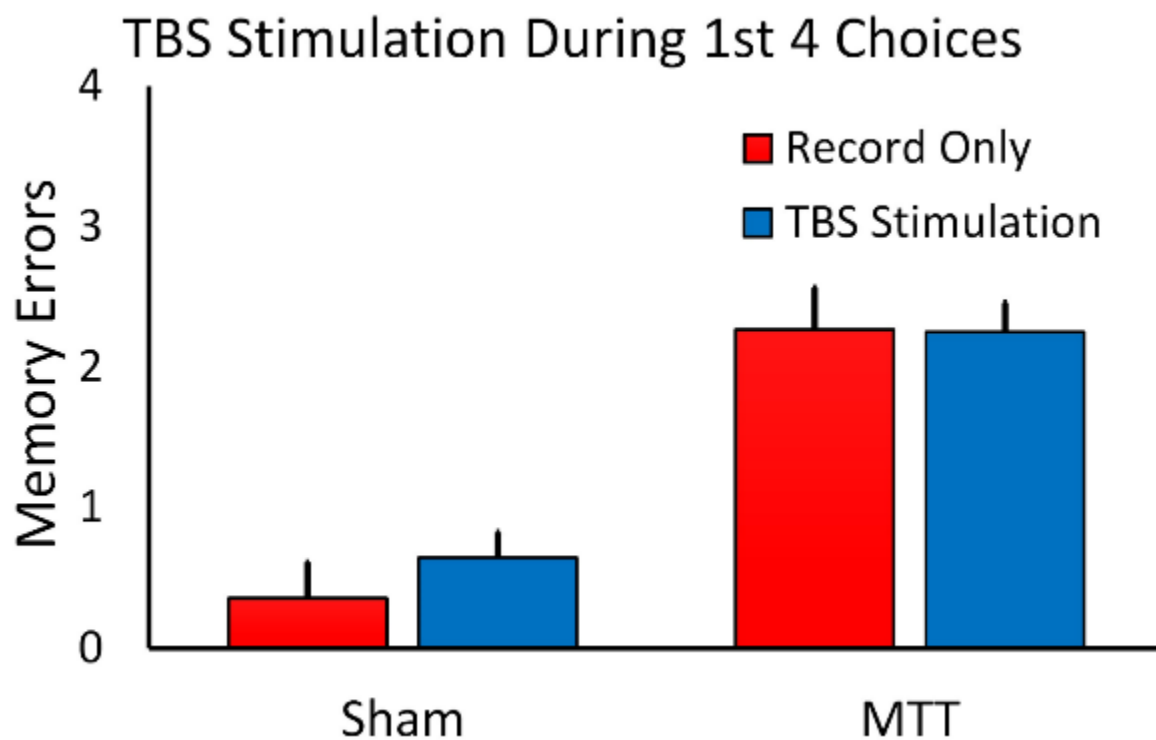


Figure 8.44: Mean + SEM spatial memory errors in the RAM for record only (red) and TBS (blue) sessions in the RAM (five days of each).

### 8.3.25. ATN TBS zif268 induction

To investigate the effect of TBS stimulation of the ATN further, rats were stimulated for 30 minutes unilaterally before a final trial in the standard RAM task and were perfused after 90 minutes in a quite dark room. The first four days of RAM testing were used to re-familiarise both groups with the procedure before and then performance spatial memory performance was compared between the last day of standard testing (day 4) and 30 mins unilateral TBS prior to testing (day 5) between days and lesion groups.. MTT lesions were still impaired relative to shams on these two final days of testing (Lesion,  $F(1,19)$ ,  $p < 0.001$ , figure 8.45), but no significant change in performance was associated with TBS stimulation (TBS and TBS x Lesion,  $p > 0.1$ )

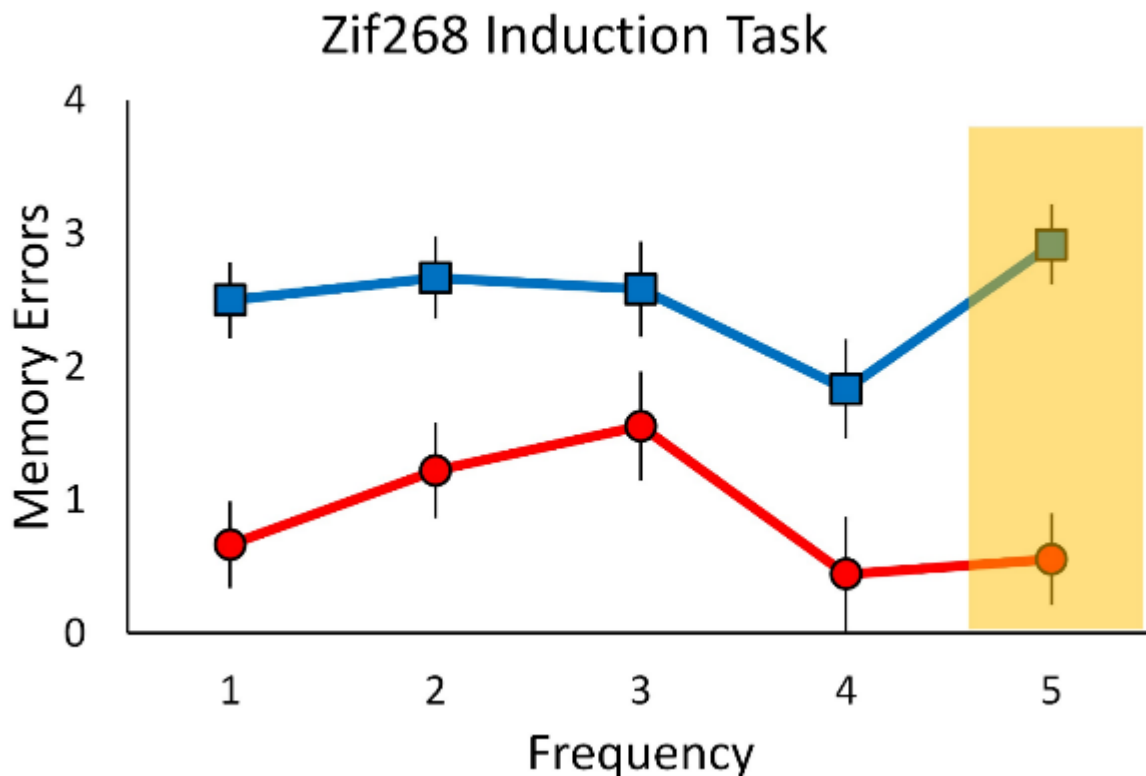


Figure 8.45: Mean  $\pm$  SEM spatial memory errors across the four days of RAM testing. On the fifth day (yellow bar) rats received 30 minutes of unilateral ATN TBS before the RAM task.

### 8.3.26. Zif268 expression after TBS

### 8.3.27. Retrosplenial cortex

Zif268 expression was analysed between the TBS and non-TBS hemispheres and across groups to determine the effect of TBS and MTT lesions on zif268 immunoreactivity in the retrosplenial cortex and dorsal hippocampus. Zif268 expression was normalised to the sham group when comparing the non-TBS hemisphere between groups, and to the non-TBS hemisphere within respective groups to determine TBS related effects. Each region of interest was analysed separately using independent t-tests for between group analyses and 2 X 2 ANOVA for TBS related changes between TBS and non-TBS hemispheres. MTT lesions reduced zif268 expression across the subregions of the retrosplenial cortex (figures 8.46 & 8.47), with the most profound changes occurring in the superficial ( $t(1,18) = 5.83, p < 0.001$ ) and deep ( $t(1,18) = 4.58, p < 0.001$ ) granular b region. Less striking zif268 reductions were also found in the superficial ( $t(1,18) = 2.33, p < 0.05$ ) and deep ( $t(1,18) = 3.39, p < 0.005$ ) dysgranular cortex. TBS stimulation did not significantly alter zif268 expression in any subregion of the retrosplenial cortex in either in the sham or MTT group ( $p > 0.1$ , figures 8.48 & 8.49).



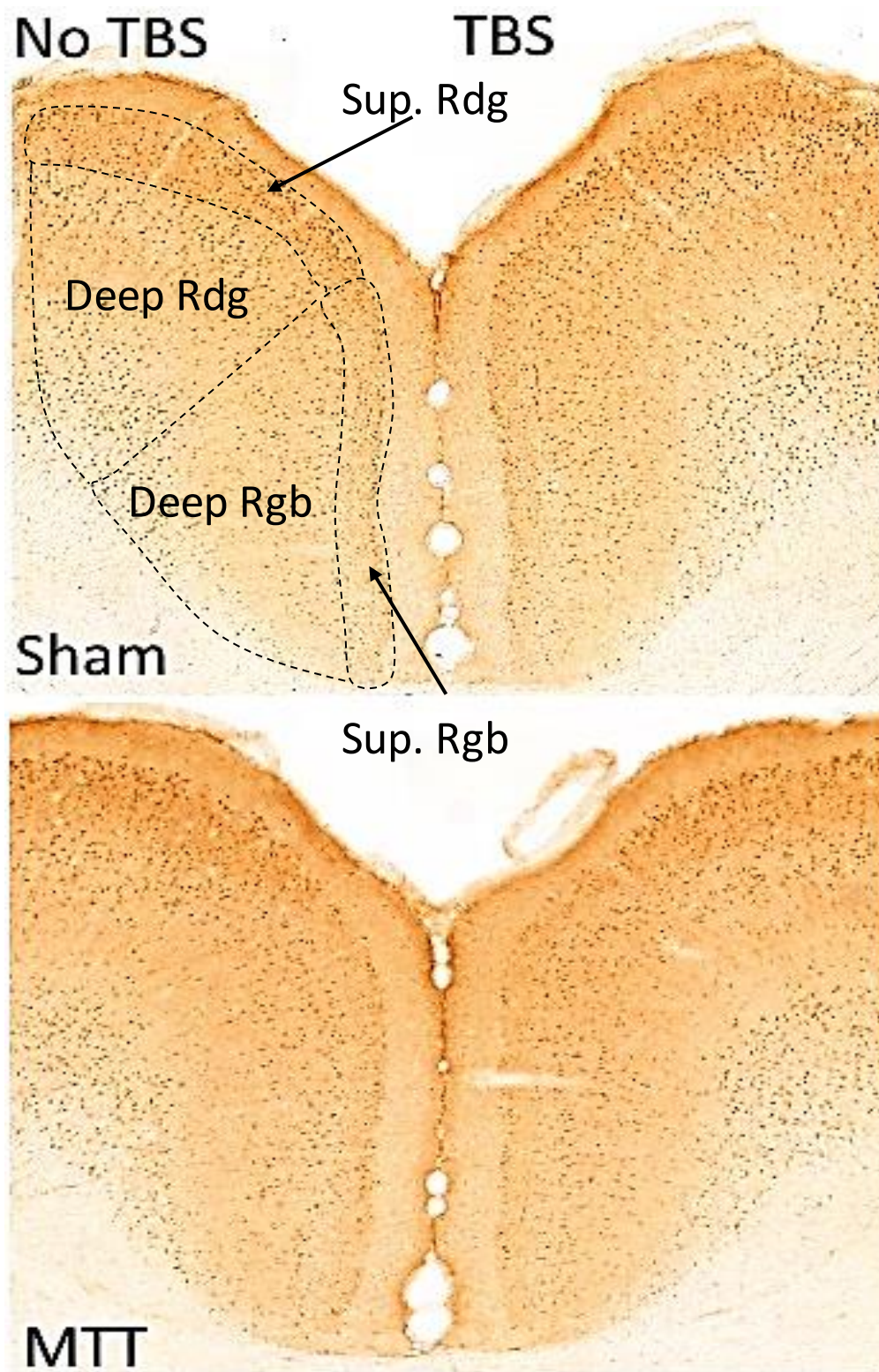


Figure 8.46: Photomicrographs of zif268 expression in the subregions of the retrosplenial cortex in the non TBS hemisphere (left) and TBS hemisphere (right) in a rat with a Sham lesion (top) and MTT lesion (bottom). Rgb = granular b retrosplenial cortex; Rdg = dysgranular retrosplenial cortex; deep = deep cortical layers; sup. = superficial cortical layers.



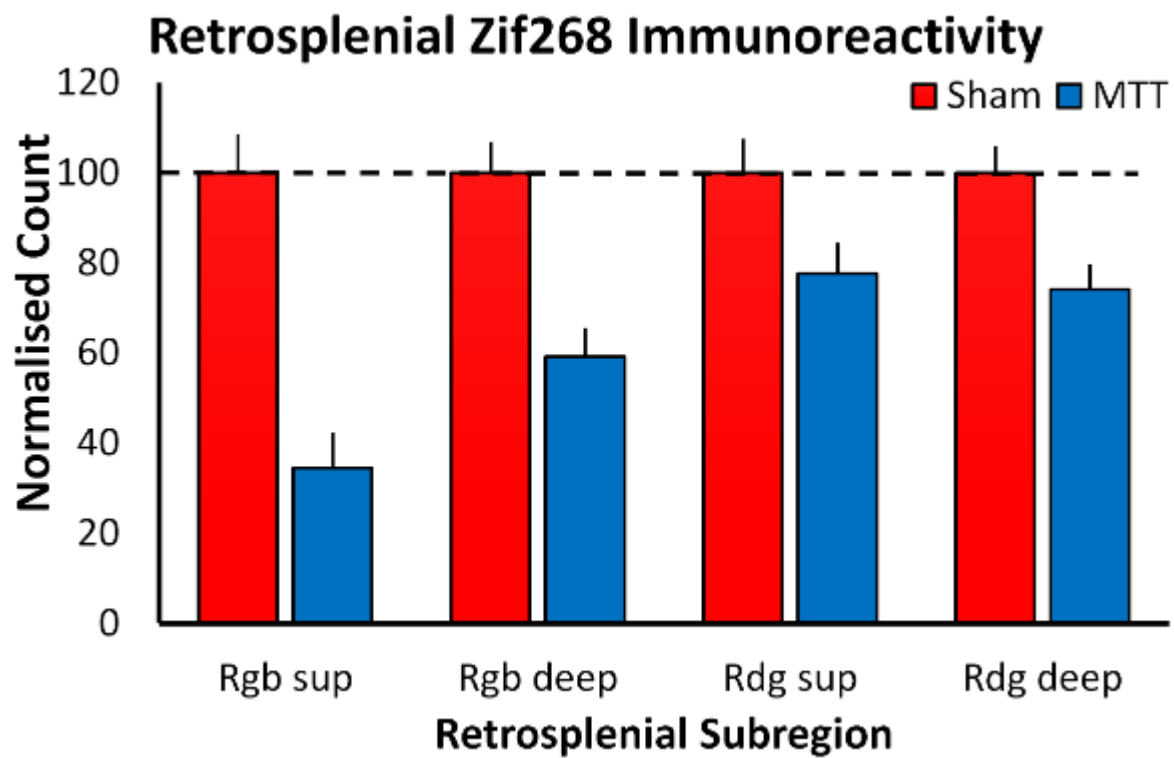


Figure 8.47: Mean + SEM normalised *zif268* counts for the Sham and MTT groups across the subregions of the retrosplenial cortex in the non-TBS hemisphere.

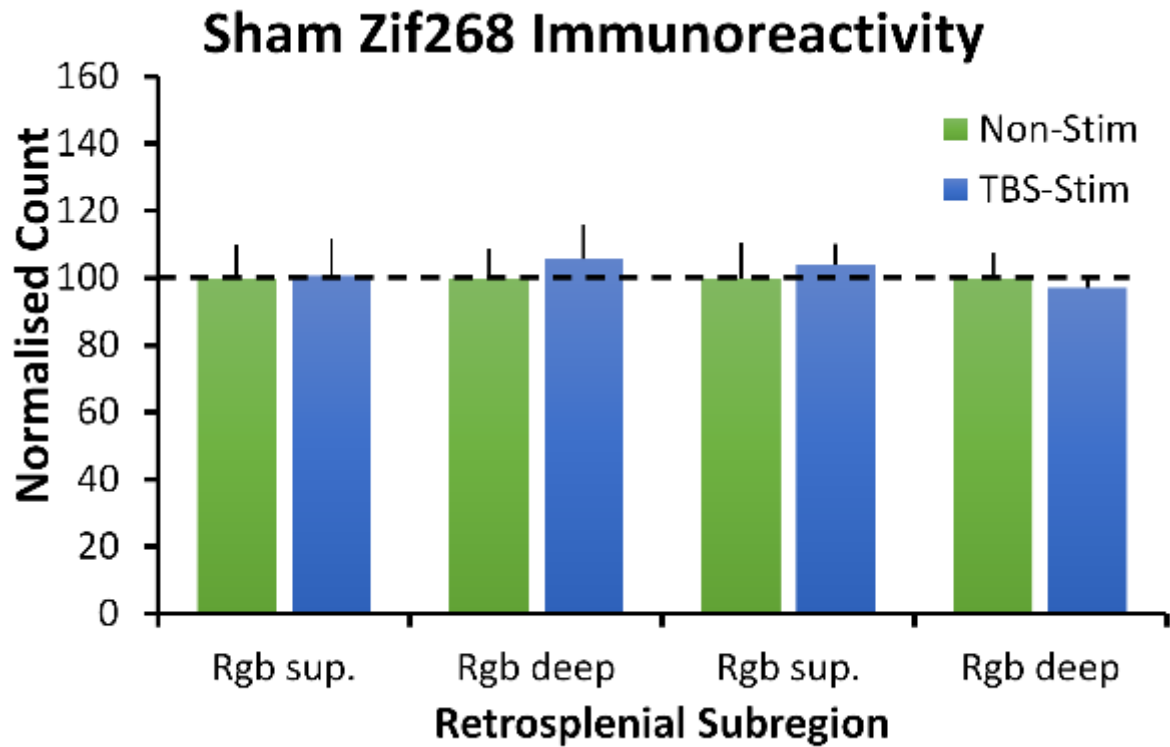


Figure 8.48: Mean + SEM normalised *zif268* counts for the Sham group between the non-TBS and TBS hemispheres across the subregions of the retrosplenial cortex.

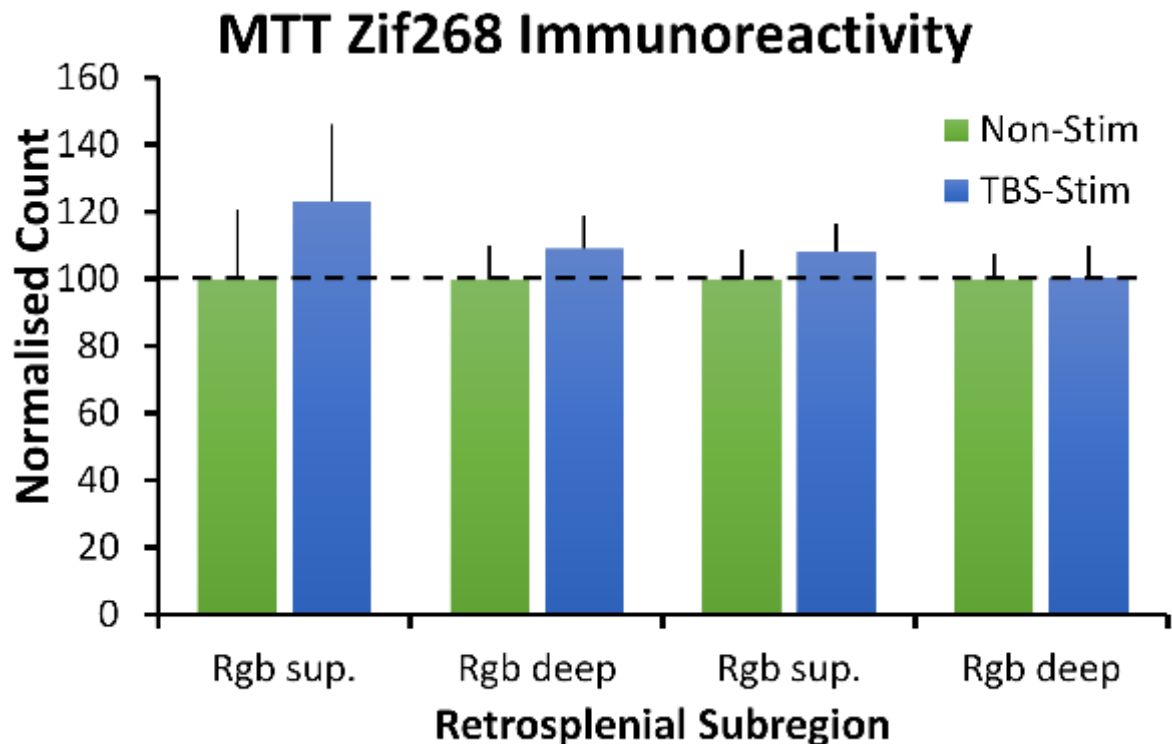


Figure 8.49: Mean + SEM normalised *zif268* counts for the MTT group between the non-TBS and TBS hemispheres across the subregions of the retrosplenial cortex.

### 8.3.28. Dorsal hippocampus

Zif268 expression in three subregions of the dorsal hippocampus, CA1, the dentate gyrus (DG) and the combined CA3/CA4 region (CA4 is sometimes referred to as the hilus of the DG; figure 8.50). MTT lesions resulted in increased zif268 expression in the CA3/4 region although this difference failed to reach significance ( $t(1,18) = -1.93, p = 0.07$ ; figure 8.51). No lesion related changes in zif268 expression were found in the CA1 or DG regions ( $p > 0.4$ ). However, TBS stimulation of the ATN significantly increased zif268 expression in the combined CA3/4 region in the Sham, but not the MTT group (Lesion,  $F(1,14) = 5.52, p = 0.03$ , figures 8.52 & 8.53) and (Stimulation  $\times$  Lesion,  $F(1,14) = 5.85, p = 0.029$ ). There was also an overall increase zif268 expression in CA1 when both lesion groups were considered together (Stimulation,  $F(1,14) = 13.02, p < 0.005$ ), but no effect of Lesion, or Lesion  $\times$  Stimulation ( $p \geq 0.07$ ). No significant differences were found for the DG ( $p > 0.07$ ).

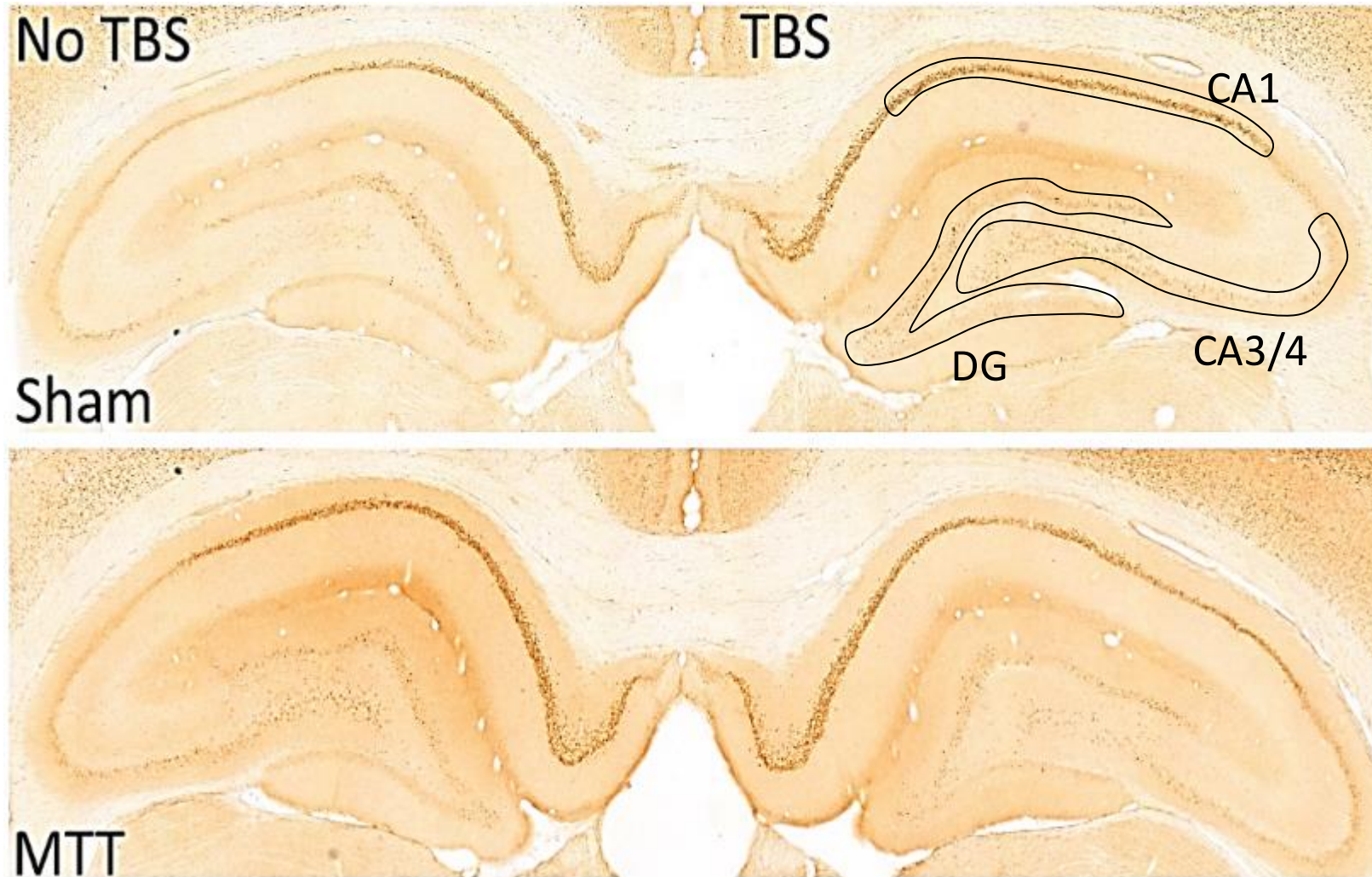


Figure 8.50: Photomicrographs of zif268 expression in the sub-regions of the dorsal hippocampus in the non TBS hemisphere (left) and TBS hemisphere (right) in a rat with a Sham (top) and a MTT lesion (bottom)

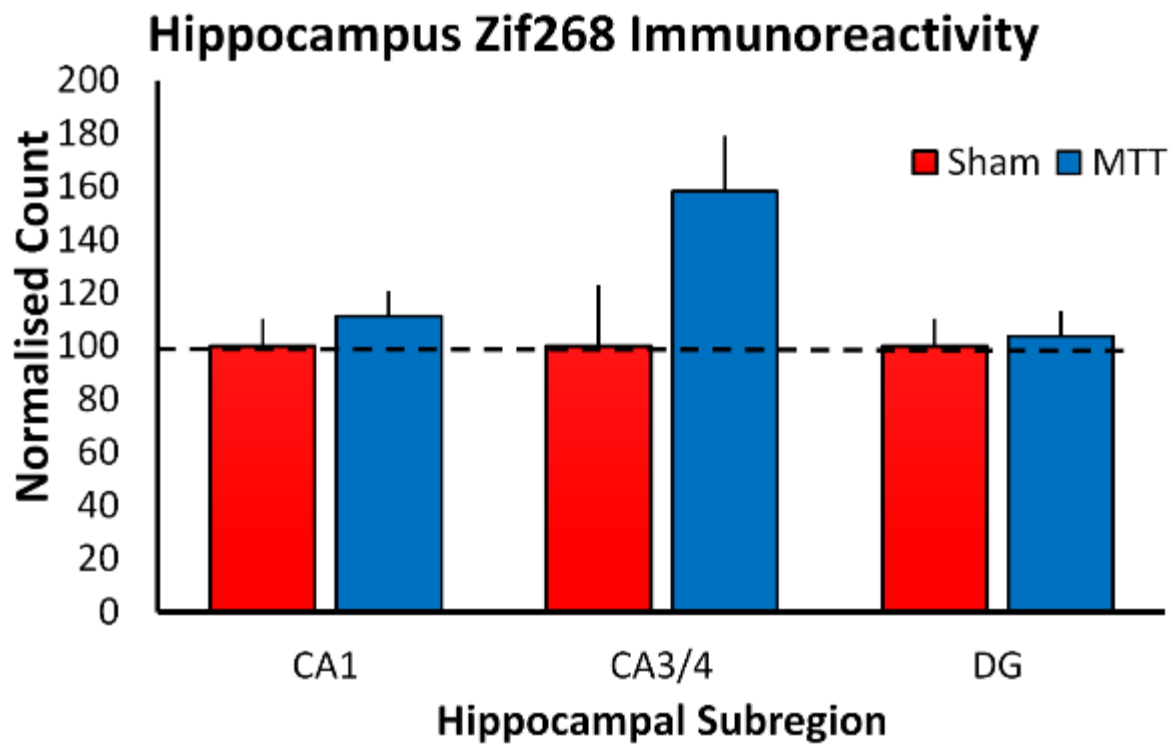


Figure 8.51: Mean + SEM normalised *zif268* counts for the Sham and MTT groups across the sub-regions of the dorsal hippocampus in the non-TBS hemisphere.

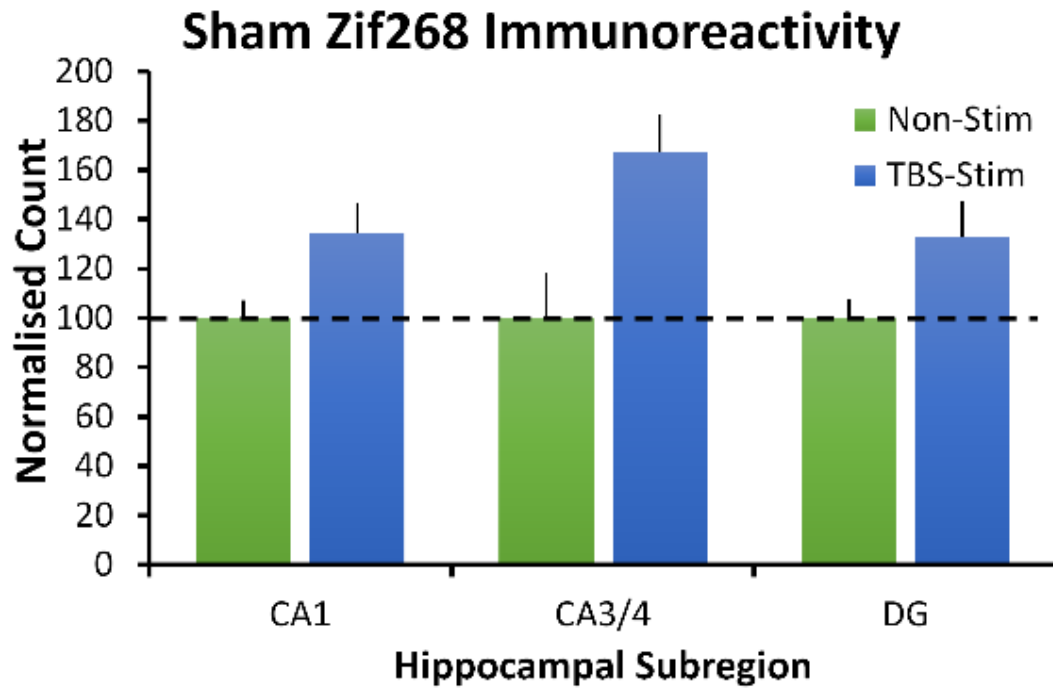


Figure 8.52: Mean + SEM normalised zif268 counts for the Sham group between the non-TBS and TBS hemispheres across the sub-regions of the dorsal hippocampus.

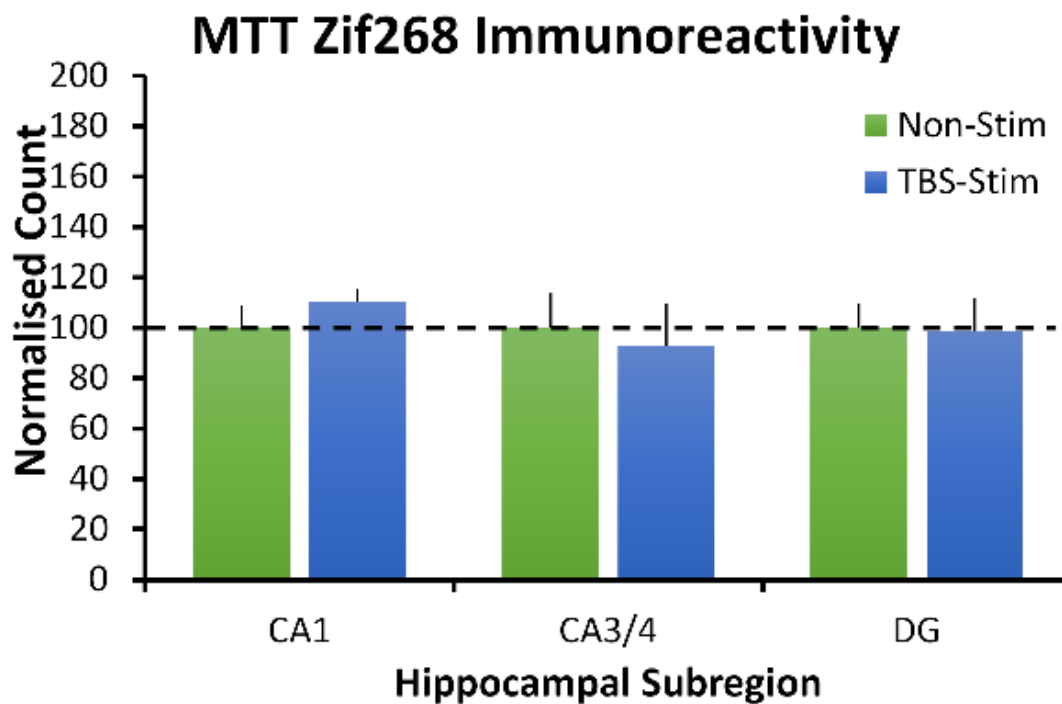


Figure 8.54: Mean + SEM normalised zif268 counts for the MTT group between the non-TBS and TBS hemispheres across the subregions of the dorsal hippocampus.

## 8.4. Discussion

This study provides the first evidence that lesions to the mammillothalamic (MTT) tract disrupt rhythmic oscillatory interactions between three critical nodes in an extended hippocampal circuit associated with episodic memory. More specifically, MTT lesions reduced spectral power and coherence within and between the hippocampus (HPC), anterior thalamus (ATN) and prefrontal cortex (PFC) under isoflurane anaesthesia and during ‘choice’ epochs across memory tasks in the radial arm maze (RAM). These findings provide direct support that the dense amnesia associated diencephalic pathology in humans may be caused by impaired interactions across a wider neural network involving the hippocampus and prefrontal cortex.

In addition to the severe working memory deficits in the radial arm maze, MTT lesions resulted in a consistent reduction in theta band coherence during correct arm choices between the ATN and PFC, with more variable changes to spectral power in the HPC and ATN and PFC. The strongest lesion related reductions in coherence were found during spatial memory performance after a 5 minute delay was added following the first four arm choices in the RAM. In this task MTT lesions profoundly impaired theta coherence between the HPC-PFC, HPC-ATN and ATN-PFC. Moreover, these lesion related differences between the HPC-PFC and ATN-PFC became more pronounced following the mid trial delay when cognitive load was at a premium. An initial exploration of theta-gamma cross frequency coupling revealed greater modulation of high-theta high-gamma interactions in the sham group during the first four correct arm choices in the beam break RAM task. MTT lesions also reduced hippocampal spectral power in the beta band and reduced beta coherence between the HPC-PFC and HPC-ATN under isoflurane anaesthesia. Novel insight was also gained in to the functional and behavioural effects of TBS to the ATN. One second bursts of TBS were sufficient to increase peak low theta power in the prefrontal cortex of sham rats, and low theta coherence between the HPC and PFC. Unilateral TBS also resulted in increased ipsilateral *zif268* expression in the dorsal hippocampus of sham rats. However, TBS during the first four arm choices in the standard RAM task had no effect on spatial memory performance. This study also replicated reports that MTT lesions result in reduced expression of *zif268* in the retrosplenial cortex, with no changes found in the dorsal hippocampus (Frizzarti et al. 2016).

The presence of altered functional activity both within and especially between the key structures of HPC, PFC and ATN, following MTT lesions, provides a highly novel contribution to work in this area. Previous research has investigated the impact of MTT lesions on static biomarkers of neural integrity and activation throughout the extended hippocampal circuit (Vann & Albasser, 2009; Mendez-Lopez et al., 2013; Dupire et al., 2013; Vann, 2013; Harland et al., 2014; Frizzarti et al., 2016). The most prominent reductions have been found in retrosplenial cortex with the immediate early genes *c-fos* and *zif268*. Like previous reports, the current study replicated the results of chapter 6 and found striking hypo-activation of *zif268* in the superficial and deep layers of the granular and dysgranular retrosplenial cortex, but not in the hippocampus following MTT lesions (Frizzarti et al., 2016). Changes in the hippocampus and prefrontal cortex have been reported previously, but these appear to be task and marker specific (Dupire et al., 2013; Dillingham et al., 2015; Dalrymple-Alford et al., 2016). One limitation of this evidence, however, is that it relies on examining isolated structures in post-mortem tissue, which constrains conclusions regarding altered or dysfunctional interactions. In the current study electrophysiology allowed the direct pairing of behaviour and neural activity within multiple distinct brain regions simultaneously.

Only two studies have examined the effect of damage to the mammillary region on rhythmic theta activity, both looking only within the hippocampus (Thinschmidt et al., 1995 & Sharp & Koester., 2008). In both of studies, however, their lesions were electrolytic and extended far beyond the MB to include the supramammillary nucleus, which itself has an established role in modulating hippocampal theta (Pan et al., 2002) and thus do not address the influence of MB and MTT lesions *per se*. One of these studies reported negligible changes in hippocampal EEG (Thinschmidt et al., 1995), and the other a 1 Hz slowing of hippocampal theta cell firing in awake behaving rats (Sharp & Koester, 2008). However, neither of these studies used a mnemonic task to drive rhythmic activity, instead recordings were made in a small arena (Thinschmidt et al., 1995), or during a random foraging task (Sharp & Koester, 2008). One prominent hypothesis of MB involvement in neural rhythmicity suggests that MB lesions would alter inter-structural communication, rather than drive hippocampal activity *per se* (Kirk & Mackay, 2003). Accordingly, the current study found that highly selective MTT lesions produced substantially reduced theta coherence between the HPC-PFC, HPC-ATN and ATN-PFC during the mid-trial delay spatial working memory task in the RAM. Importantly, no differences between the groups in either power or



coherence were observed during the 5 minute mid-trial delay period, suggesting these changes were related to mnemonic processing rather than exposure to a spatial environment per se. Less consistent reductions in coherence and spectral power both within and between the HPC, PFC and ATN were observed in the MTT group for the standard RAM tasks. It appears that dysfunctional interactions between the ATN and PFC may be a hallmark of MTT related pathology because reduced ATN-PFC coherence was observed across all RAM tasks and in the random foraging task that had no task specific demands. Consistent with a cognitive impact for this deficit, recent evidence has suggested that ATN-cortical interactions in the theta band might be critical for successful memory encoding in humans (Sweeney-Reed et al., 2014).

The reduced HPC-PFC theta coherence observed in the midtrial delay task in rats with MTT lesions is consistent with previous studies that have suggested a critical role for hippocampal-prefrontal theta synchrony during successful spatial memory processing in rodents (Jones et al., 2005; Hyman et al., 2010; Kim et al., 2011; O'Neill et al., 2013). Like previous studies, sham rats had consistent peaks in HPC-PFC theta coherence during correct arm choices across RAM testing. By contrast, rats with MTT lesions typically had flatter HPC-PFC theta coherence within these same choice epochs. Additional evidence supporting a critical role for HPC-PFC interactions came from a comparison of correct and incorrect trials during the first LFP RAM task. For this task the sham group had increased HPC-PFC theta coherence when they made correct arm choices, whereas no change in theta coherence was apparent in the MTT group. It appears then that the MTT group may be failing to process spatial information correctly even when they are not making spatial errors. Further support for this position was found in both the mid trial delay and beam break RAM task. In these tasks early trials where both groups performed accurately were contrasted with late trials, when MTT rats made considerably more spatial errors than the sham group. Interestingly, the MTT related reductions in coherence on these tasks were present in the early trials on the beam break RAM task along with a reduction in high-theta high-gamma cross-frequency coupling. These findings are suggestive of a spatial encoding deficit, which is consistent with previous behavioural evidence (chapter 4) suggesting MTT lesions are especially sensitive to tasks requiring the rapid encoding of spatial information (Dillingham et al., 2015)

EEG monitoring under general anaesthesia has often been employed to assess in real time the functional state of the central nervous system (Rampil, 1998). Furthermore, tight control over parameters such as depth of anaesthesia and the cessation of motor responses make this a useful tool for investigating rhythmic activity following brain damage. Therefore, an initial investigation of the impact of MTT lesions on circuit wide rhythmic activity was made following electrode implantation. Consistent with previous work isoflurane anaesthesia resulted in a general slowing of rhythmic activity compared to awake active states (i.e. random foraging) regardless of lesion status (Mashour et al., 2010; Hudetz et al., 2011; Maciver & Bland, 2014). This was particularly apparent in the ATN and PFC, both of which had the greatest spectral power within the delta band. By contrast, there were two peaks in spectral power in the hippocampus one in the delta band and another in the beta band, which is also consistent with a previous report (Hudetz et al., 2011). Comparing across groups MTT lesions resulted in a substantial reduction in hippocampal beta power relative to sham rats. Furthermore, MTT lesions also reduced beta coherence between the HPC-ATN and HPC-PFC. This altered activity must have occurred via the ATN, either directly via the cingulum bundle or indirectly via the retrosplenial cortex because the MTT only directly innervates the ATN, and not other structures in the extended hippocampal system (Kirk & Mackay, 2003; Dillingham et al., 2015).

The application of a tail pinch appears to have reduced beta and increased delta spectral power in the hippocampus for the sham group, with negligible effect on hippocampal oscillatory activity in the MTT lesion group. Tail pinch has been used previously to induce rhythmic theta activity in various brain structures in anaesthetised rats (Kocsis & Vertes, 1994; Kirk et al., 1993; Kocsis & Vertes, 2001). These studies, however, used urethane to anaesthetise their animals. Urethane has the advantage of minimal effects on cardiovascular and respiratory systems whilst maintaining spinal reflexes (Hara & Harris, 2002). By contrast, isoflurane administration at concentrations above 1.8% has been shown to totally abolish reflexive withdrawal and substantially reduced activity in spinal neurons (Jinks et al., 2002). Therefore, a stronger tail pinch response may have been elicited with a lower concentration of isoflurane. Nonetheless, these findings still provided novel evidence that MTT lesions disrupt rhythmic oscillatory activity in critical nodes of the extended hippocampal system.

One possible explanation for the changes observed in rhythmic oscillatory activity in anaesthetised and awake behaving rats with MTT lesions is altered acetylcholine activity. MB lesions have been shown to alter cholinergic activity in the cortex and hippocampus and reduce ACh binding to muscarinic receptors in the ATN (Sikes & Vogt, 1986; Berocochea et al., 1995). Within the hippocampus, ACh release is thought to be driven primarily by activation of cholinergic neurons located in the medial septum/diagonal band (MS/DB). The MS/DB is able to modulate hippocampal excitability through the interactions of ACh, GABA and glutamate (Savage et al., 2012). As a result ACh receptor activation in the hippocampus can lead to a number of synchronised oscillatory states, which depend on ACh concentration and the subtype of the ACh receptor that is activated (Drever et al., 2011). Savage et al. (2012) have suggested that the ability of the diencephalon to alter hippocampal ACh activity might relate to hypoactivity in the retrosplenial cortex (RSC), which in turn reduces feedback to the MS/DB. It is well established that MTT lesions can result in profound functional changes in the RSC indicated by hypoexpression of the immediate early genes *zif268* and *c-fos* (Vann & Albasser, 2009; Vann, 2013; Frizzarti et al., 2016). In addition, a study injecting the neural tracer horse radish peroxidase found evidence that the MS/DB neurons are reciprocally innervated by the RSC providing a mechanism for these changes to occur (Gonzalo-Ruiz and Morte, 2000).

Like previous studies, large MTT lesions resulted in a severe spatial working memory impairments in the radial arm maze (Vann & Aggleton, 2003, Vann, 2013; Nelson & Vann, 2014). The current study demonstrated that this impairment persists for an extended period of post-surgery training, although there was some evidence of improved performance in the MTT group towards the end of testing. Surprisingly, the addition of a five minute delay after the first four arm choices did not further impair the performance of the MTT group despite additional task demands. Previously, the addition of a one minute mid-trial delay or a midtrial delay with maze rotation in the RAM were found to exacerbate the MTT deficit (Vann & Aggleton 2003, Vann, 2013, Chapter 6). Maze rotation has been proposed to make intramaze cues (such as odour trails) incongruent with extramaze spatial information and remove a possible performance strategy (Pouthuzian et al., 2008). One possibility for the poorer RAM performance in the current study by comparison to that found in Chapter 6 is that the rats in this electrophysiology study were only familiarised to the RAM prior to surgery whereas

explicit training was given in the earlier study. Consistent with this explanation, Warburton et al., (1999) found that training rats before ATN and fornix lesions facilitated the reacquisition of a fixed platform location in the water maze, although both lesion groups were still impaired relative to shams.

These results also provide clarity in to the considerable uncertainty surrounding the origins of theta rhythmicity within the diencephalon (Dillingham et al., 2015). In rodents, neither the MB nor ATN possess interneurons (Wang et al., 1999; Dillingham et al., 2015). For this reason they are unable to produce the inhibitory component necessary to generate rhythmic oscillations, supporting the notion that rhythmicity within these structures is driven by their afferent connections (Tsanov, 2015). For the ATN this could be either via ascending inputs from the MTT, or direct hippocampal innervation provided by the fornix (Tsanov, 2015). If the MTT was primarily driving rhythmic activity in the ATN then MTT lesions would be expected to have a profound impact on the rhythmic oscillatory activity in the ATN. For example, inactivation of the medial septum, which is a critical rhythmic input into the hippocampus, resulted in a profound reduction in hippocampal theta rhythmicity (McNaughton et al., 2006). However, in the present study MTT lesions had a negligible impact on rhythmic activity in the ATN under anaesthesia, during random foraging or spatial working memory tasks in the RAM. These results suggest that rhythmic activity within the ATN is most likely driven by its hippocampal inputs. Consistent with this finding, Tsanov et al. (2011c) reported that low frequency stimulation of the hippocampal, but not the MB inputs into the ATN were able to increase the amplitude of ATN theta spectral power. By contrast it appears the MTT inputs may have more of a role in increasing ATN excitability rather than rhythmically entraining its neurons. For example, transection of the mammillothalamic tract in rabbits reduced spontaneous baseline unit activity in the anteroventral nucleus of the ATN (Gabriel et al., 1995). Whilst in another study, high frequency stimulation of the MTT, but not the fornix, was able to induced large amplitude and stable long term potentiation of the ATN field response (Tsanov et al, 2011d).

However, the comments immediately above do not answer the question of the functional role of MB rhythmicity within the extended hippocampal circuit. The results of the current study appear to be consistent with Kirk and Mackay's (2003) recurrent theta network model. In this scenario, theta rhythmicity propagated by the MB/MTT would provide a mechanism to facilitate the alignment of rhythmic activity in the diencephalon, hippocampal

formation and neocortex during mnemonic processing. Therefore, the loss of diencephalic rhythmicity would be predicted to disrupt neural synchrony rather than the frequency or amplitude of neural oscillations. As would be expected, MTT lesions were found to primarily impact theta coherence between the HPC, PFC and ATN, and these effects were strongest in the midtrial delay RAM task when demands on memory were high. However, in their model Kirk and Mackay (2003) implied that hippocampal inputs were solely responsible for driving theta rhythmicity in the MB. More recent evidence suggests that MB inputs from the VTg are also important (Vann, 2013). Like the ATN, the MB also receives a dense input from the hippocampus via the fornix, but critically also receives a unique non-hippocampal theta input from the VTg (Kocsis et al., 2001; Vann & Nelson, 2015). Separate recording studies have provided evidence supporting role for both of these MB inputs in local rhythmic activity (Kocsis & Vertes, 1994; Kirk et al., 1996; Kocsis et al., 2001; Bassat & Poindessous-Jazat, 2001). However, behavioural lesion studies using spatial working memory tasks have strongly suggested that it is the loss of VTg inputs, and not loss of the post commissural fornix (pFx) projection to the MB, that contributes to the spatial memory deficit found after MB or MTT lesions (Vann, 2009; Vann et al., 2011; Vann, 2013). In these studies VTg lesions produced similar memory deficits and retrosplenial and hippocampal c-fos reductions to MTT lesions (Vann, 2009; Vann, 2013). By contrast, lesions to the pFx had negligible effects on spatial memory or c-fos expression (Vann et al., 2011; Vann, 2013). Further research is necessary to fully explore the contribution of the VTg to theta rhythmicity in the MB. To address this issue recordings from the ATN, HPC and PFC could be taken from rats with VTg and pFx lesions during spatial memory tasks, or local field and unit recording could be made from the medial MB of rats with these same lesions.

A secondary goal of this work was to assess the suitability of theta burst stimulation (TBS) to the ATN to enhance memory performance. TBS was used here because it has previously been shown to increase long term potentiation in the hippocampus (Bowden et al., 2012). Bilateral theta burst stimulation of the ATN had slight modulatory effects on spectral power in the prefrontal cortex and increased low range theta coherence between the HPC and PFC in the sham group. These findings are somewhat consistent with previous animal work showing that electrical stimulation of the ATN below 40Hz was able to modulate the hippocampal field potential (Styckopolski et al., 2014). Furthermore, a more recent study

showed that relatively high voltage (~8V) stimulation of the ATN increased activity, measured with a BOLD response, in the parahippocampal gyrus, hippocampus and prefrontal cortex (Gibson et al., 2016). A comparison of *zif268* expression between the stimulated and non-stimulated hemisphere suggests that in the sham group unilateral ATN TBS upregulated hippocampal activity in the CA3/4 region. Like the present findings a previous study found that 100Hz stimulation of the ATN increased *c-fos* expression in the CA3 region of the dorsal hippocampus relative to non-stimulated shams (Hescham et al., 2015).

Furthermore high frequency stimulation of the ATN has also been shown to increase hippocampal neurogenesis (Toda et al., 2008; Hamani et al., 2011; Encinas et al., 2011), which improved memory performance at a later time point (Hamani et al., 2011). However, here no changes in spatial memory performance were observed for either the MTT or sham group following TBS stimulation given during the first four arm choices. In previous studies, high frequency stimulation of the ATN (100 Hz +) impaired spatial alternation in the t-maze and failed to enhance object discrimination in rats treated with scopolamine (Hamani et al., 2010; Hescham et al., 2015). The lack of enhanced memory performance observed in the current study could relate to the relatively brief stimulation period. That is, the 90 secs (max) of stimulation was not sufficient to appropriately activate the circuit. Alternately, it might be that the ~5V used here was not sufficient to drive activity within the hippocampus and prefrontal cortex. It is also possible that a different stimulation protocol might be more relevant. Further research is necessary to determine optimal stimulation parameters that might be used to improve rhythmic coherence between the HPC, ATN and PFC following MTT lesions.

One limitation of the present study was that because sham rats quickly acquired the RAM task and maintained an accurate level of performance so that electrophysiological data from incorrect trials in this group was limited, especially in the later RAM tasks. Future work could address this issue by introducing a 12 arm RAM, which would provide more choice data and a more difficult task to elicit more errors in the sham group. Alternately, an object-place paired associate task could also provide significant insight into lesion related dysfunctional interactions because it would allow a longitudinal perspective of electrophysiological changes both within and between subjects. It would also be of considerable interest to examine the impact of other diencephalic lesions to rhythmic activity. Of particular importance would be to examine the impact of ATN lesions on interactions

between the HPC, PFC and the retrosplenial cortex. Further research should also again study the possibility of memory enhancement via stimulation of the ATN. The relatively compact size of the ATN along with its dense cortical and hippocampal connections make it a prime candidate for therapeutic interventions. For example, optogenetics could be used to selectively target the glutamatergic projection neurons in the ATN. Then a physiologically relevant stimulation protocol could be delivered based on activity from an intact animal or real time feedback from the HPC or PFC. Similarly, our lab has produced evidence that enriched housing and systemic neurotrophic-like peptides enhance memory performance in rats with ATN lesions, and partially restore spine counts in the CA1, but do not re-establish IEG activity or reverse spine loss in the RSC (Dupire et al., 2013; Harland et al., 2014; Loukavenko et al., 2016). We have some initial collaborative results with colleagues at Otago, using ATN lesions, that coherence between the HPC and PFC during random foraging may benefit from enrichment (Ulrich et al, in prep). Looking at electrophysiology as per the current study, with a sensitive RAM task, may be especially informative in this regard.

The findings of the present study provide a significant contribution to our current understanding of the role of rhythmic oscillatory activity within the diencephalon. We have provided the first evidence that aberrant rhythmic activity can occur within an extended neural network following localised MTT lesions. Furthermore, dysfunctional interactions between the HPC, PFC and ATN in the MTT lesion group were more pronounced during the mid-trial delay RAM task, which placed greater demands on mnemonic processing. These findings have wider significance because degeneration of this same neural network is responsible for the profound episodic memory loss found in Alzheimer's disease and suggest that localised neuropathological changes that begin early in the ATN regions in this disorder (Aggleton et al, 2016) can have a profound impact on distal regions. The role of the diencephalon in theta rhythmicity has also been further elaborated. It seems likely that the hippocampal, not MB inputs, are driving rhythmic activity within the ATN. Rather, in agreement with Kirk and Mackay's (2003) proposal rhythmic activity in the diencephalon, especially within the theta band appears to contribute to the regulation of synchrony between the diencephalon, hippocampus and prefrontal cortex during memory-relevant tasks. However, it is likely that the unique brainstem influence from the VTg also contributes to this process. Furthermore the present results reinforce the importance of rhythmic interactions between the HPC and PFC during spatial memory processing. In support of this contention,

the less accurate MTT lesion rats had reduced HPC-PFC theta coherence during correct arm choices and only sham rats showed increased HPC-PFC theta coherence in correct vs incorrect choices in the RAM.



# Chapter 9

## General discussion

### 9.1. Summary of findings

The current study has summarized the current research on clinical and experimental studies of diencephalic amnesia, and provided novel empirical evidence in particular on the functional impact of MTT lesions on an extended hippocampal system for episodic memory. The MTT provide a dense unidirectional innervation to the ATN from the MB (Dillingham et al., 2015) suggesting the MTT may have a critical and profound influence on ATN functioning and thereby the extended hippocampal memory system (Aggleton, 2014; Vann & Nelson, 2015). If this were the case, then it would be expected that MTT lesions would result in at least equivalent behavioural and functional deficits compared to those found after ATN lesions. This hypothesis was tested for the first time in a major experimental study described in Chapter 6. That study of a direct comparison between the effect of MTT lesions and ATN lesions revealed that MTT lesions generally produced less severe memory deficits across spatial tasks in the water-maze and radial arm maze. This difference was especially pronounced when reference memory was examined in the classic version of the water maze task. That is, ATN lesions produced marked deficits in spatial reference memory in the water maze, in line with previous reports, whereas rats with MTT lesions did not differ from shams. Furthermore, ATN lesions also resulted in greater reductions of Zif268 expression in the retrosplenial cortex and hippocampal CA1 than was found after MTT lesions. The lesser impact of MTT lesions across these measures suggests that additional dense connections of the ATN, including reciprocal connections with the retrosplenial cortex and hippocampal formation, and afferent connections with the prefrontal cortex, have a functional influence that additional to that provided by information supplied by the MTT to the ATN. Further support for this contention came from evidence that MTT lesions profoundly reduced NeuN positive cell counts across the two medial and lateral subdivisions of the MB, whereas a similar, yet milder reduction was found in the ATN group. If MB input was critical for ATN function then the greater impact of MTT lesions on MB integrity should have produced at least equivalent if not more severe deficits than those produced by ATN lesions.

Another, important novel finding was provided by the second empirical chapter. Here, MTT lesions were shown to impair the acquisition of object-place paired associate learning in a task that required bi-directional conditioning. Unlike reference memory in the water maze, reference memory can be impaired after MTT lesions when a conditional spatial discrimination is required. Previous work has reported that ATN lesions substantially impair acquisition of a similar task, but there was, surprisingly, no influence of MB lesions in this context (Sziklas et al., 1996; Sziklas & Petrides, 1999). The ability to form arbitrary associations between a stimulus and a context, such as an object and a place, is a fundamental aspect of episodic memory that would be expected to be disrupted by both MB and MTT lesions, the latter because the MTT appears to carry only fibre afferents from the MB to the ATN. We used a new task that could test the possibility that directional learning, perhaps subsumed by head direction cells and pathways separate to that supplied by the MB, might explain previous failure to find an object-place impairment with MB lesions. Specifically, the inclusion of probe trials provided certainty that both groups were associating a rewarded object with a specific location, and did not rely on direction, egocentric strategies or local odour cues to solve the task. While it remains possible that subtle procedural differences explain the difference between the previous failure to find place-object associations after MB lesions (Sziklas et al., 1996) and the current deficit with MTT lesions, the important point is that a significant anomaly has been resolved. Our finding is consistent with one report that MTT lesions produced an impairment in spontaneous object-in-place recognition, a working memory measure of an object-place association (Nelson & Vann, 2014).

Furthermore, the findings of distal dysfunction within a wider neural circuit found in the first experiment were extended by investigating the impact of MTT lesions on rhythmic oscillatory activity in the extended hippocampal memory system during performance of a spatial memory task. This electrophysiological evidence is, to our knowledge, the first of its kind in the literature. The addition of electrophysiology allowed real time functional interactions within and between the hippocampus, ATN and prefrontal cortex, three critical nodes of the circuit. Arguably the most novel finding, from a functional perspective, was that MTT lesions resulted in reduced HPC-PFC, HPC-ATN and ATN-PFC coherence in the upper theta band (8-12 Hz) during a correct choices in mid-trial delay RAM task. Reductions in hippocampal theta and beta and ATN gamma power as well as ATN-PFC theta coherence were also observed in the MTT group during the first LFP recording task in the standard

RAM task. Furthermore, when correct and incorrect choices on this task were compared sham, but not MTT rats showed increased HPC-PFC theta coherence during correct choices. Recent evidence has also suggested that theta-gamma cross-frequency coupling may be critically involved in mnemonic processing (Tort et al., 2009; Sweeney-Reed et al., 2014; Heusser et al., 2016). An initial investigation into theta-gamma cross-frequency coupling found stronger interactions between high-theta phase and high gamma amplitude in the sham group during the first four correct arm choices on the standard RAM task with beam breaks. The most consistent finding was reduced ATN-PFC coherence in the MTT group across all LFP recording RAM tasks, also replicated in the random foraging task. MTT lesions also reduced hippocampal beta power and beta coherence between the HPC-ATN and HPC-PFC under isoflurane anaesthesia where parameters could be tightly regulated across animals. Replicating the results of the first experiment, MTT lesions reliably impaired spatial working memory performance in the RAM across testing, and also resulted in a striking reduction of Zif268 expression in the RSC in the non-TBS stimulated hemisphere. However, the reduction previously observed in hippocampal CA1 was not replicated when comparing across non-TBS hemispheres.

An investigation of the potential for theta burst stimulation of the ATN to enhance memory function also produced some interesting findings. In sham rats, TBS increased low theta power (4-8 Hz) in the prefrontal cortex; it also decreased delta coherence but increased low theta coherence between the HPC-PFC. In the MTT group TBS only reduced high theta coherence between the HPC-PFC. Interestingly, bilateral TBS stimulation of the ATN did not affect spatial working memory performance in the RAM for the sham or MTT group. Another interesting finding was that unilateral TBS stimulation increased overall Zif268 expression in hippocampal CA1 across both groups when comparing the TBS to non-TBS hemispheres, but only significantly increased expression in hippocampal CA3/4 in the sham group. No Zif268 changes were found in the retrosplenial cortex after TBS of the ATN.

Discussion of each of the above sets of empirical findings and their theoretical value is presented below, followed by a consideration of the limitations of the current study and future directions.

## 9.2. Comparative MTT lesion and ATN lesion effects on spatial memory

Like previous studies, MTT lesions consistently produced spatial working memory deficits in the water maze and radial arm maze (Vann & Aggleton 2003; Vann, 2013; Nelson & Vann, 2014). However, the MTT lesion working memory impairments found here were less severe than those observed for ATN lesions, especially in the RAM. Interestingly, the MTT lesion deficit observed in the post-lesion (before electrode implantation) standard RAM testing in the third experiment was more pronounced than the MTT deficit found in experiment one. One explanation for this discrepancy is that rats with MTT lesions in the first experiment were able to retain some of what they learnt during preoperative RAM training to reduce reducing the postoperative deficit. By contrast, in experiment three rats were only habituated to the maze before lesion surgery. Consistent with this explanation, Warburton et al. (1999) found that rats with fornix or localised ATN lesions trained preoperatively on a reference memory task in the water maze showed some re-acquisition of this task post lesion, but remained impaired compared to sham rats.

Another point of difference between the results of expt 1 and expt 3 was the effect of MTT lesions after the addition of mid-trial delay in the RAM. The addition of a one minute delay following the first four arm choices in expt 1 significantly increased spatial memory errors in the MTT group, whereas no change in MTT performance was apparent in expt 3 following the addition of a five minute delay. It is possible this related to the absence of preoperative training and / or the more open RAM that was used in expt 3. This new RAM configuration was used to enable electrophysiological recording. Unlike the RAM used in exp 1, the new RAM configuration had all the door guides and pullies located underneath the maze allowing the rats an unrestricted view of the spatial environment, which may have facilitated their performance. Furthermore, unlike expt 1, the rats were contained within the maze during the 5 minute delay, which may have provided less disruption to the rats.

Spatial reference memory in the water maze provided a critical point of difference between ATN and MTT lesions in the present study. ATN lesions replicated earlier findings of a severe reference memory impairment in this task (Warburton et al., 1999; Wilton et al., 2001; Wolff et al., 2007; 2008). By contrast, the MTT lesion group performed equivalent to shams, which was inconsistent with one previous report although those impairments were mild at best (Winter et al., 2011). In line with the present findings, separate studies from the

same lab found that ATN, but not MTT lesions impaired the ability of rats to learn a geometric discrimination in a modified water-maze task (Aggleton et al., 2009; Dumont et al., 2013; Vann, 2013).

### 9.3. MTT lesions impaired object-place associations

In addition to spatial working memory, the ability to form arbitrary associations between a stimulus and a context is a fundamental aspect of episodic memory (Preston & Eichenbaum, 2010). Previous studies have shown that the hippocampus, prefrontal cortex and ATN are all critical to this process when spatial location defines the correct item when one or two stimuli are used (Sziklas et al., 1996; Sziklas & Petrides, 1999; Gilbert & Kesner, 2002; Kesner & Ragozzino, 2003; Browning et al., 2005; Gibb et al., 2006; Dumont et al., 2014). However, contrary to what would be expected, rats with extensive MB lesions had equivalent performance to controls on an object-place association task (Sziklas et al., 1996). This finding presents an anomaly within the literature and suggests a clear distinction between ATN and MB lesions that required revisiting, which may reflect intact reference memory after MB dysfunction and or the ability of rats with MB lesions to use direction to solve the task.

In the current study, MTT lesions which provide a more selective removal of the MB in terms of its medial versus lateral components, impaired acquisition of object place pairings in a diamond maze across an extended training period. Consistent with these findings, a recent study found that MTT lesions impaired spontaneous (but working memory) object in place discriminations, and impaired acquisition of a spatial location when directional cues were rendered irrelevant (Nelson & Vann, 2014). The ability to use directional information to select between the rewarded and unrewarded object may explain the discrepancy between the present findings and those of Sziklas et al. (1996). That is, because trials were run from either end of an open field rats could have learnt that object A is correct if approached from directly direction A, and object B is correct if approached from direction B. In the present study a large open diamond arena may have discouraged directional strategies and probe tests confirmed that performance did not rely on the use of directional, egocentric or odour cues to make accurate spatial-object associations in shams and in MTT rats. The MTT lesion deficit reported here is milder than those observed following ATN, hippocampal or crossed unilateral ATN-hippocampal lesions used in earlier studies (Sziklas et al., 1996; Sziklas et al., 1999; Henry et al., 2004; Dumont et al., 2010). Lesions to either or a combination of these two structures profoundly impaired acquisition of object-place associations often with no

improvement observed, even after 500 trials. Interestingly, rats with fornix lesions were unimpaired on the task used by Sziklas et al. (1998), which in the context of absent effects after their MB lesions suggested to those authors that non-fornical pathways between the ATN and hippocampus via the retrosplenial cortex might be critical (Sziklas et al., 1999). Therefore it is possible that preserved ATN-retrosplenial interactions reduced the severity of the MTT deficit on this task. A direct comparison between the MTT and ATN was not made on this task because the ATN and ATN sham group were undergoing alternative testing as part of a different (enrichment) study by a different student, although there is clear impetus for doing such a comparison.

#### 9.4. The impact of MTT lesions on the extended hippocampal circuit

Previous studies have consistently shown that both MTT and ATN lesions result in striking reductions of the immediate early genes, *zif268* and *c-fos* in cortical regions, but the most striking and consistent changes have been found in the retrosplenial cortex, especially the superficial Rgb layer (Jenkins et al 2002 a,b; Jenkins et al., 2004; Poirier & Aggleton 2009; Vann & Albasser 2009; Dupire et al., 2013; Vann 2013; Loukavenko et al., 2016; Frizzarti et al., 2016). Additional Rgb retrosplenial changes have been observed following MTT and ATN lesions in the metabolic marker cytochrome oxidase, suggesting retrosplenial dysfunction is a cardinal feature of lesions to the limbic diencephalon (Frizzarti et al., 2016; Medez-Lopez et al., 2013). The retrosplenial cortex, has dense reciprocal connections with both the ATN and hippocampal formation, and as such is thought to provide a critical convergence point between the medial diencephalon and medial temporal lobe. Less consistent reductions of IEG markers have been observed in the hippocampus suggesting that changes in this region may be both marker and task specific (Jenkins 2002; Dumont et al 2012; Vann & Albasser, 2009; Frizzarti et al., 2016; Loukavenko et al., 2016). Spatial working memory deficits following MTT and ATN lesion are thought to in part reflect these functional changes in distal sites (Aggleton & Nelson, 2015). Consistent with this view, the current study showed that both MTT and ATN lesions resulted in a striking loss of *zif268* expression in the superficial layers of the Rgb, with more modest reductions found in hippocampal CA1. In addition, the severity and extent of these *zif268* reductions paralleled the magnitude of spatial working memory performance in each group at least in the RAM. For example, in the MTT group the reduced *zif268* expression was restricted to the superficial Rgb, with a mild reduction also observed in CA1. By contrast, in the more

severely impaired ATN group zif268 reductions were found in both the superficial and deep Rgb, the superficial Rdg and superficial Rga as well as a greater reduction in CA1. The more profound zif268 reductions found after ATN lesions possibly related to the different anatomical connectivity of these two regions. The MTT can only impact the function of distal regions via its unidirectional influence on the ATN and, unlike the ATN, the MTT changes do not reflect direct deafferentation of the RSC or hippocampal subiculum (Vann & Albasser, 2009; Wright et al., 2010; Aggleton, 2012). The profound loss of zif268 expression in the superficial Rgb observed here was replicated in the third experiment, with a further reduction in zif268 expression in the deep Rgb and superficial and deep layers of the Rdg. However, unlike expt 1 MTT lesions did not reduce zif268 expression in CA1, the dentate gyrus or CA3/4 of the hippocampus. Previous work has shown that ATN related reductions in c-fos expression in the retrosplenial cortex become more pronounced with increased time intervals following lesion surgery (Poirier & Aggleton, 2009). However, both expt1 and expt 3 had approximately the same survival time following MTT lesion surgery, therefore this explanation is unlikely and is also unable to account for the different pattern of results in the hippocampus. The tasks used to induce zif268 expression were vastly different between experiments. Zif268 expression, especially in the hippocampus is known to be heavily influenced by spatial memory tasks (Guzowski et al., 2001; Poirier et al., 2008; Farina & Commins 2016). In expt 1, a massed midtrial rotation and delay task in the RAM was used to drive activity within the extended hippocampal circuit and make zif268 expression in the RSC more behaviourally relevant (Vann & Aggleton, 2002; 2004; Pothuizen et al., 2008). Although, rats were also tested on a spatial memory task in the RAM in expt 3, the primary aim in this experiment was to examine the effect of unilateral ATN stimulation on Zif268 expression in the hippocampus and prefrontal cortex. Therefore, the differences between experiments may relate to differences in the zif268 induction procedure used.

Both MTT and ATN lesions also had a profound impact on the integrity of the MB. Previous studies have consistently reported MB atrophy, and suggested probable neural loss following examination of nissl stained sections following MTT lesions in rats (Vann & Aggleton, 2003; Vann, 2013). Previous research in monkeys has also shown that lesions to the anterior, but not posterior medial thalamus results in substantial degeneration and gliosis in the MB (Aggleton & Mishkin 1983). The current study provided the first convincing evidence that MTT and ATN lesions result in neural atrophy in the MB by examining the

neural specific marker NeuN. MTT lesions resulted in a drastic reduction of NeuN positive counts in both the medial and lateral subdivisions of the MB. The same pattern of results was found following ATN lesions but to a lesser degree. The reduced NeuN counts were especially pronounced the pars lateralis of the medial MB in both groups. The loss of MB neurons following MTT or ATN lesions is not unexpected because in the central nervous system an axotomy or the removal of the primary projection target of an axon often induces a progressive functional decline in the originating cell, causing cellular atrophy and eventually cell death (Barron, 2004).

Reduced NeuN positive cell counts in the lateral MB following MTT lesions was an unexpected finding. A previous retrograde tracer study suggested that MTT lesions primarily disconnected the medial mammillary nuclei from the anteromedial and anteroventral thalamic nuclei without disrupting lateral MB anterodorsal thalamic nuclei connections, which suggested that MTT lesions leave the lateral MB relatively intact (Vann & Albasser 2009). One possible explanation for this discrepancy between the current study and with previous work is slight differences in lesion methodology, as the relative anterior-posterior location, depth or laterality of the lesion may be an important factor in the resulting impact on the MB.

Looking across the comparative effects of MTT and ATN lesions discussed far it appears likely that MTT inputs provide an important contribution to ATN function. However, lesions to the MTT do not replicate ATN lesion effects on spatial memory, or distal dysfunction within the extended hippocampal circuit implying that additional cortical and hippocampal connections to the ATN are also important.

As discussed above, previous work has examined biomarkers of neural function following MTT lesions and reported profound changes in regions distal to the injury site, such as the retrosplenial cortex. One limitation with these studies is that these markers are examined in post-mortem tissue in isolated structures and provide little insight in to altered or dysfunctional interactions that may be taking place within the extended hippocampal circuit during behaviour. Therefore electrophysiology was used here to gain additional insight in to the wider impact of MTT lesions by recording from three critical nodes in the extended hippocampal circuit, namely the ATN, hippocampus and prefrontal cortex during spatial memory in the RAM. Previous work has suggested that rhythmic interactions between the HPC-PFC, especially within the theta band, are critical for spatial working memory



performance in rats (Jones & Wilson, 2005; Hyman et al., 2013, O'Neill et al., 2011). Consistent with these findings MTT lesions resulted in significant theta decoupling between the HPC-PFC whilst making a correct arm selection in a mid-trial delay RAM task. Theta decoupling was also observed between the HPC-ATN and ATN-PFC on this task. Additional evidence supporting a critical role for HPC-PFC interactions came from a comparison of correct and incorrect choice trials in the first LFP recording RAM task. Rats with sham lesions showed a substantial decoupling of HPC-PFC theta coherence on incorrect choice trials. By contrast, HPC-PFC theta coherence was not differentially effected by incorrect trials in rats with MTT lesions, suggesting a key deficit after MTT lesions. Changes in ATN and hippocampal power were also observed in the first LFP recording RAM task and the beam break RAM task although these effects were less consistent. MTT lesions, however, also produced a consistent reduction in ATN-PFC theta coherence in both the RAM tasks and during a random foraging task in an open arena. This finding suggests that dysfunctional ATN-PFC interactions may be a hallmark of distal impairments following MTT lesions. Such a conclusion resonates with recent evidence in humans has suggested a critical role for ATN-neocortical theta interactions during encoding (Sweeney-Reed et al., 2014).

Surprisingly, MTT lesions had minimal effects on rhythmic oscillatory activity within the ATN, either under anaesthesia or in awake behaving rats. If MB inputs were critical for driving rhythmic activity within the ATN, then it would be expected that MTT lesions would dramatically attenuate ATN rhythmic activity. These results suggest that rhythmic activity within the ATN, may instead be driven by its direct hippocampal inputs. Consistent with this finding, Tsanov et al. (2011c) reported that low frequency stimulation of the hippocampal, but not the MB inputs into the ATN were able to increase the amplitude of ATN theta spectral power. By contrast, it appears the MTT inputs may have more of a role in increasing ATN excitability rather than rhythmically entraining its neurons. For example, transection of the mammillothalamic tract in rabbits reduced spontaneous baseline unit activity in the anteroventral nucleus of the ATN (Gabriel et al., 1995). In another study, high frequency stimulation of the MTT, but not the fornix, was able to induced large amplitude and stable long term potentiation of the ATN field response (Tsanov et al, 2011d).

The current electrophysiology findings appear to be consistent with Kirk and Mackay's (2003) recurrent theta network model. In this scenario, theta rhythmicity propagated by the MB/MTT would provide a mechanism to facilitate the alignment of

rhythmic activity in the diencephalon, hippocampal formation and neocortex during mnemonic processing. Therefore, the loss of diencephalic rhythmicity would be predicted to disrupt neural synchrony rather than the frequency or amplitude of neural oscillations. As would be expected, MTT lesions were found to primarily impact theta coherence between rather than power within the HPC, PFC and ATN, and these effects were especially pronounced in the midtrial delay RAM task when demands on memory were high. However, in their model Kirk and Mackay (2003) imply that hippocampal inputs are solely responsible for driving theta rhythmicity in the MB. More recent evidence suggests that MB inputs from the VTg are also important (Vann, 2013). Like the ATN, the MB also receives a dense input from the hippocampus via the fornix, but critically also receives a unique non-hippocampal theta input from the VTg (Kocsis et al., 2001; Vann & Nelson, 2015). Separate recording studies have provided evidence supporting a role for both of these MB inputs in local rhythmic activity (Kirk et al., 1996; Kocsis & Vertes, 1994; Kocsis et al., 2001; Bassat & Poindessous-Jazat, 2001). However, behavioural lesion studies using spatial working memory tasks have strongly suggested that it is the loss of VTg inputs, and not loss of the post commissural fornix projection to the MB, that contributes to the spatial memory deficit found after MB or MTT lesions (Vann, 2009; Vann et al., 2010; Vann, 2013). In these studies VTg lesions produced similar memory deficits and similar retrosplenial and hippocampal c-fos reductions to MTT lesions (Vann, 2009; Vann, 2013). By contrast, lesions to the postcommisural fornix had negligible effects on spatial memory or c-fos expression (Vann et al., 2010; Vann, 2013).

## 9.5. MTT lesions and TBS of the ATN

A secondary goal of this work was to assess the suitability of theta burst stimulation (TBS) to the ATN to enhance memory performance. The relatively small size of the ATN in addition to its dense connections to multiple cortical and subcortical regions suggest it may provide a suitable target for deep brain stimulation for memory enhancement. Considerable work has already examined the efficacy of high frequency ATN stimulation to prevent seizure activity in rats (Toda et al., 2008; Hamani et al., 2011; Encinas et al., 2011). TBS was used here because it has previously been shown to increase long term potentiation in the hippocampus, and many cells within the AV fire in theta bursts (Tsanov et al., 2011a; Bowden et al., 2012). Bilateral theta burst stimulation of the ATN had slight modulatory effects on spectral power in the prefrontal cortex and increased low range theta coherence between the HPC and PFC

in the sham group. These findings are somewhat consistent with previous animal work showing that tonic electrical stimulation of the ATN in an sheep model below 40Hz was able to modulate the hippocampal field potential, whereas frequencies above 40Hz suppressed hippocampal activity (Stykolowski et al., 2014). Furthermore, a more recent study showed that relatively high voltage (~8V) stimulation of the ATN increased activity, measured with a BOLD response, in the parahippocampal gyrus, hippocampus and prefrontal cortex in a porcine model (Gibson et al., 2016). A comparison of Zif268 expression between the stimulated and non-stimulated hemisphere suggests that in the sham group unilateral ATN TBS upregulated hippocampal activity in the CA3/4 region. Like the present findings a previous study found that 100Hz stimulation of the ATN increased c-fos expression in the CA3 region of the dorsal hippocampus compared to non-stimulated controls (Hescham et al., 2015).

Another interesting finding is that high frequency stimulation (100 + Hz ) of the ATN increases hippocampal neurogenesis (Toda et al., 2008; Hamani et al., 2011; Encinas et al., 2011), which improved memory performance at a later time point (Hamani et al., 2011). However, here no changes in spatial memory performance were observed for either the MTT or sham group following TBS stimulation given during the first four arm choices in the RAM. In previous studies, high frequency stimulation of the ATN (100 Hz +) impaired spatial alternation in the T-maze and failed to enhance object discrimination in rats treated with scopolamine (Hamani et al., 2010; Hescham et al., 2015). The lack of enhanced memory performance observed in the current study could relate to the relatively brief stimulation period. That is the 90 secs (max) of stimulation was not sufficient to appropriately activate the circuit. Alternately, it might be that the ~5V used here was not sufficient to drive activity within the hippocampus and prefrontal cortex. It is also possible that a different stimulation protocol might be more relevant. Further research is necessary to determine optimal stimulation parameters that might be used to improve rhythmic coherence between the HPC, ATN and PFC following MTT lesions.

## 9.6 Limitations of the current study

The first experiment in the current study was run in parallel with an ATN enrichment study, run by another student, which shared the standard housed ATN and ATN sham group. This

made both studies more efficient sharing the workload and maximising the amount of information gained from the rats used. However, this meant that the ATN-MTT lesion comparisons were not possible for the object-place association task. Nonetheless, previous work has provided a clear and consistent deficit for ATN lesions on paired-associate learning tasks (Sziklas & Petrides, 1999; Gibb et al., 2006; Dumont et al., 2014), with uncertainty only surrounding the role of the MB in for this type of task (Sziklas et al., 1996). In this respect the current study provided a significant contribution to this literature presenting convincing evidence that MTT lesions impair the acquisition of object-place associations. Clearly, a direct comparison between ATN lesions and MTT lesions on object-place memory would be needed to determine the relative effects of these lesions.

Examining NeuN and Zif268 positive cell counts was sufficient to provide a relative measure of lesion related changes in key regions. It may also be informative, however, to employ additional techniques. For example, future work could use unbiased stereology to provide estimates of total NeuN positive cell counts within the medial and lateral subdivisions of the MB between groups. Alternately, western blotting or RNA assays could be used to give relative levels of Zif268 or NeuN concentrations. One drawback of the latter methods, however, is that it is difficult to examine subregions within a structures because relatively large amounts of tissue are required to capture sufficient protein/RNA to analyse. For example, Guzowski et al. (2001) examined relative RNA concentrations of Zif268, c-fos and Arc (all IEGs) associated with learning within the entire hippocampus. However, evidence from the current study and earlier work suggests there are clear differences in Zif268 expression across both the hippocampal and retrosplenial subregions that may be obscured if these were aggregated (Poirier et al., 2008; Farina & Commins, 2016; Frizzarti et al., 2016).

It would also have been informative to record from additional regions such as the RSC or anterior cingulate in the current study. However, practical considerations limited the amount of electrodes that could be implanted within a rat brain. One approach might be to use multiple electrode arrays with contacts spaced along the probe to capture multiple structures, or multiple regions within a structure (Ulrich et al; in prep). However, one concern with this approach is producing excessive collateral damage, especially within sensitive regions with multiple small nuclei known to be involved in mnemonic processing within areas such as the thalamus. This could be minimised by using silicon probes (Buzsaki, 2002),

which can be tens of micrometres thick and can support up to 64 channels of recording. However, these are currently prohibitively expensive and at present are best used in acute recording experiments.

A further limitation of the present study was that the accurate performance of the sham lesion rats in the RAM task provided few error trials during expt 3. For this reason electrophysiological correlates of successful and unsuccessful memory processing could only be examined in the first LFP recording RAM task. Previous studies have suggested that theta coherence between the HPC-PFC was associated with successful spatial memory processing in rats (Jones & Wilson; Hyman et al., 2010). The results of the LFP recording RAM task were consistent with this with lower HPC-PFC theta coherence during incorrect arm choices found in the sham rats. Future work could use a more difficult task such as a 12 arm RAM, which provides more opportunities for sham rats to make incorrect arm choices. Furthermore, an alternative and more difficult task such learning during object-place paired association could be used. Because this task has a long acquisition period changes in rhythmic activity could be explored across different stages of task acquisition both within and between groups.

### 9.7. Future directions

It would be of considerable interest to further examine the interactions between different frequency bands that occurred during choice points in the RAM. Recent evidence from both humans and animals has suggested that cross-frequency coupling, especially between the theta and gamma bands plays a critical role in mnemonic processing (Tort et al., 2009; Colgin et al., 2011; 2013, Heusser et al., 2016). An initial investigation of cross-frequency coupling within the hippocampus between theta phase and gamma amplitude was examined for the 5 minute delay RAM task. A further comparison between hippocampal theta phase and ATN and PFC gamma amplitude is also planned. Furthermore, the impact of incorrect trials, at least in the MTT group could also be examined across structures and tasks.

Although the current study has provided major new evidence, further research is needed to understand the contribution of the diencephalon to rhythmic oscillatory activity with the extended hippocampal memory circuit. For example it is still not clear whether the hippocampal, VTg or a combination of both inputs are driving rhythmic activity in the MB. New research could examine the impact of VTg and post commissural fornix lesions on MB rhythmic activity and unit firing, together with their impact on structures upstream in the

extended memory system. Furthermore, an obvious next step would also be to examine the effect of ATN lesions on rhythmic oscillatory activity within the hippocampus and prefrontal cortex during spatial memory tasks, so that we can compare those findings with those provided in this thesis. An initial study, Ulrich et al. (in prep) found that ATN lesions were associated with a reduction in prefrontal theta power and a modest reduction in low theta power in the dentate gyrus from the first to a second trial of a random foraging task. However, it would be expected that driving mnemonic processing during a more active memory task would have more profound effects. As mentioned previously, the retrosplenial cortex appears to be especially sensitive to lesions to the diencephalon (Aggleton & Neslon, 2015; Dillingham et al., 2015). The retrosplenial cortex is also a critical site of pathology in Alzheimer's disease, often showing altered function early in disease progression (Aggleton et al., 2016). Therefore, additional recording electrodes placed within the RSC could provide critical insight in to altered or dysfunctional interactions between the HPC, RSC and PFC following ATN lesions.

Evidence has also suggested a mnemonic role for additional thalamic nuclei such as the mediodorsal nucleus (MD) and its contribution to memory remains unresolved despite considerable effort (Mitchell & Dalrymple-Alford, 2005; Mitchell & Dalrymple-Alford, 2006; Aggleton et al., 2011; Pergola & Suchan, 2013; Dalrymple-Alford et al., 2015; Mitchell & Chakraborty, 2015). The MD has dense connections with the prefrontal cortex and cortical regions of the temporal lobe (Mitchell & Chakraborty, 2015). Evidence suggests that this thalamo-cortical circuit may synchronise within the beta frequency range and play an important complimentary role providing executive control over the extended hippocampal theta circuit (Ketzer et al., 2015). Furthermore, animal evidence suggests that these circuits are involved in different aspects of declarative memory. The extended hippocampal circuit has been strongly implicated in recollection, whilst the MD-cortical circuit may be preferentially involved in familiarity processes (Aggleton et al., 2010; Aggleton et al., 2011). Further work could examine how these two regions interact during memory tasks to form integrated and cohesive memory traces. For example, lesions could be made to the ATN and MD and recording electrodes implanted in the PFC, HPC and temporal cortex as well as the non-lesioned thalamic structure in respective groups. Rats could then be run on a series of tasks that involve recollection, recognition or both. This could be further improved by using

temporary inactivation of either structure with optogenetics, which would allow for within group comparisons.

Similarly, the nucleus reuniens (Re) of the thalamus has received recent interest because of its dense connections with both the hippocampus and prefrontal cortex (Cassel et al., 2013). In fact the reuniens contains neurons that bifurcate to innervate both structures (Hoover & Vertes, 2012). This suggests that the Re may have a role in modulating hippocampal-prefrontal interactions, which are known to be important during successful memory processing (Colgin, 2011, 2013). Therefore the Re presents itself as another promising target for therapeutic intervention. Consistent with this proposal, a recent study showed that abnormal slow wave delta activity introduced to the Re via optogenetic stimulation impaired a hippocampal dependent spatial working memory task in the T-maze (Duan et al., 2015). Perhaps optogenetics could be used to specifically target the collateral neurons within the Re following lesions to the ATN or MTT. Different stimulation patterns could be implemented in many different thalamic structures to determine which frequency and pattern of stimulation has the greatest impact. Further sophistication could be added to this design by using physiologically relevant stimulation patterns recorded from an intact animal or by using a closed loop design. In a closed loop design real time neural activity from the hippocampus and prefrontal cortex could be used constantly adjust stimulation in thalamic nuclei (see Siegle & Wilson, 2014).

## 9.8. Summary

In summary, the current thesis provided several novel insights into the functional impact of MTT lesions on an extended hippocampal network for episodic memory. Clearly, MTT lesions have a profound impact on memory performance and we have the beginnings of an understanding on the impact of such lesions on the electrophysiology of a distributed network of structures involved in memory. These findings include the first direct comparison between MTT and ATN lesions and showed that ATN lesions produced more profound spatial memory deficits in the RAM and water maze. Furthermore, MTT lesions had a weaker impact than ATN lesions on Zif268 expression in the retrosplenial cortex. Our findings provide critical insight into two different perspectives regarding the critical site of pathology in diencephalic amnesia. Specifically, rather than being driven primarily by VTg inputs via the MB/MTT, it appears that the dense cortical and hippocampal inputs received by the ATN make it relatively more critical point of dysfunction than would be expected by a focus only

on the albeit important brainstem inputs to the extended memory system. Further evidence on this point was provided by the more modest effects of ATN lesions on MB neural counts, and the minimal effect that MTT lesions had on rhythmic oscillatory activity within the ATN itself. Nonetheless, MTT lesions resulted in consistent spatial working memory deficits in the RAM, and produced substantial theta decoupling between the HPC-PFC, HPC-ATN and ATN-PFC that may help explain the impact of such pathology in the context of clinical amnesia. Rather than driving ATN function, the current findings instead provided the first direct support for Kirk & Mackay's (2003) proposal that the rhythmic input from the MB/MTT to the ATN provides a mechanism to synchronise activity within the diencephalon, hippocampus and neocortex during mnemonic processing. Further research investigating altered or dysfunctional interactions between additional cortical and hippocampal regions following diencephalic damage is required to better understand the dense amnesia that can result from various kinds of diencephalic pathology in humans.



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